

1996

Chemical Effects on Microbial and Sensory Qualities of Catfish in Planktonic and Dynamic Systems.

Michael Adrain Land

Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation

Land, Michael Adrain, "Chemical Effects on Microbial and Sensory Qualities of Catfish in Planktonic and Dynamic Systems." (1996). *LSU Historical Dissertations and Theses*. 6352.
https://digitalcommons.lsu.edu/gradschool_disstheses/6352

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

CHEMICAL EFFECTS ON MICROBIAL AND SENSORY QUALITIES OF
CATFISH IN PLANKTONIC AND DYNAMIC SYSTEMS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by

Michael Adrain Land

B.S. Louisiana State University, 1984

M.S. Louisiana State University, 1988

December 1996

UMI Number: 9720366

UMI Microform 9720366
Copyright 1997, by UMI Company. All rights reserved.

**This microform edition is protected against unauthorized
copying under Title 17, United States Code.**

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

DEDICATION

This work is dedicated to my grandmother, Nelvyn Conlay, and grandfather, Adrain D. Land Sr., whose determination, vision and love brought us so far and so close.

ACKNOWLEDGMENTS

Sincere appreciation and thanks are extended to my mentor, major professor and most importantly my friend, Dr. Robert M. Grodner. His time, wisdom, gentle humor and trust in my ability has always given me the belief that all was possible.

Appreciation is also extended to other Committee Members: Dr. Joseph Liuzzo for his good natured cajoling of any situation; to Dr. James Farr who always had a kind word and a positive spin on a situation; to Dr. Paul Wilson who generally has the best grip on reality of anyone on campus; Dr. Robert Edling who was kind enough to sit in and really helped in any way he could; and finally Dr. Ramu Rao whose cooperation was vital and given in good cheer at the last moment. A special debt of gratitude is honored to Chancellor Rousse Caffey. Dr. Caffey's belief and sponsorship of my academic career was a true gift.

Special thanks to all my friends in the Food Science Department who helped in any way they could. Their biggest help was always just being there. I and the department can never repay Lizzy Escort for what

she has given us all: her time, energy, thoughts, humor and her love. Additionally, this could not have been accomplished without my Karma twin Dr. Linda Douglas. A better partner to trudge through hell with - one could not have asked for.

Thanks to all my teacher friends: Pat, Pat, Kathy, Phyllis, Jane, Glen and Linda Gail. Who would have thought sarcasm and tears would have been such a wonderful combination. And to the Jujutisu clan who have been there since high school: Ginger, Bill, Ted, Wiley, Dave, L.J., Miss Flo and Jimmy; thanks for so many things.

My deepest thanks goes out to Dr. Linda Andrews. Our plight through the last decade has been one of the best friendship building sagas told by LSU. The impetus for so much of my work and the department's growth has been inspired by Dr. Andrews.

But most importantly, I would like to thank all of my family. Their love and prayers over the long years and miles have made the burden lighter and the fight worth while. Finally - with out God, none of these blessings would have been possible.

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGMENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABSTRACT	xii
CHAPTER 1	1
INTRODUCTION	1
CHAPTER 2	7
REVIEW OF LITERATURE.	7
History of man & food.	7
Historical appreciation & development of preservation	8
Population & seafood demand increasage . . .	10
Fish population decline & eco-pyramid depletion	12
Aquaculture history	15
Aquaculture today	16
Catfish an aquaculture success story. .	19
General fish anatomy & flora	20
Microorganisms	25
Microbes & temperature	27
Microbes & food spoilage.	28
Microbial adhesion.	29
Microbes & pH	32
Fish protein	33
Sensory analysis	37
Discrimination testing.	38
Electron microscope.	39
Stainless steel	41

Chemical Agents	43
Chlorine	43
Organic acids	47
Acetic acid	49
Lactic acid	53
Peroxides	54
Survival curves & D-Values	57
<i>Listeria monocytogenes</i>	58
General information	58
Diagnosis of human illness	61
Associated foods	61
Disease frequency	61
Target population	62
Food analysis	63
Recent outbreaks	63
 CHAPTER 3	 65
LETHALITY OF CHEMICALS TO <i>Listeria monocytogenes</i>	
Scott A: D-VALUE DETERMINATIONS IN TSP AND	
CATFISH FILLETS UTILIZING 2% LACTIC ACID,	
2% ACETIC ACID, 1% HYDROGEN PEROXIDE &	
50ppm SODIUM HYPOCHLORITE	65
Introduction	65
Materials and methods	69
Broth & culture preparation	69
Catfish preparation & treatment	70
Treatment procedures and microbial	
analysis	70
Chemical preparation	71
Results and discussion	72
D-Value determinations	72
 CHAPTER 4	 79
EXTENDING SHELF LIFE OF CATFISH FILLETS ON ICE	
USING HOT HYDROGEN PEROXIDE, ACETIC OR LACTIC ACID	
ALONE AND IN COMBINATION & THEIR EFFECTS ON	
<i>Listeria monocytogenes</i>	79
Introduction	79
Materials and methods	84
Catfish preparation and treatment	84
Microbial analysis	85
Measurement of pH	86

Preparation of culture	86
Results and discussion	86
CHAPTER 5	98
SENSORY ACCEPTABILITY OF 65°C ACETIC ACID DIPPED	
CATFISH FILLETS	98
Introduction	98
Materials and methods	99
Fillet preparation and treatment	99
Panelist selection	100
Triangle test design	100
Analysis	102
Results and discussion	102
CHAPTER 6	106
SANITIZER EFFICACY AGAINST <i>Listeria monocytogenes</i>	
TO STAINLESS STEEL, POLYPROPYLENE AND POLYETHYLENE	
IN A BLOOD BIOFILM	106
Introduction	106
Materials and methods	109
Preparation of cultures.	109
Preparation of test surfaces.	109
Soiling procedures	110
Sanitizer	110
Enumeration methodology	110
Results and discussion	111
REFERENCES	116
VITA	130

LIST OF TABLES

1. Pathogens isolated from fresh water fish . . .	22
2. Mean counts of potentially pathogenic bacteria in water, sediment and fish viscera from catfish ponds	23
3. Dissociation constants of organic acids in aqueous solutions	48
4. D-Values for <i>L. monocytogenes</i> at different concentrations and chemical additives	73
5. Comparison of mean pH values for various treatments for catfish fillets stored on ice at 4°C over 12 days	87
6. Comparison of shelf life extension over 12 days with different chemical treatments, mean log APC	91
7. Comparison of <i>Listeria monocytogenes</i> suppression on inoculated fillets stored over time on ice by chemical treatment by day.	92
8. Hunter color mean value scores for L, a & b. .	104
9. CFU/mm ² of 3 processing surfaces after incubation and sanitation	111

LIST OF FIGURES

1.	World population growth curve 1961-1994 . . .	11
2.	World fish catch 1961-1994	14
3.	U.S. fish & marine fish catch 1961-1994	15
4.	Nitrogenous waste bio-remediation schematic . .	24
5.	Lateral and dorsal view of fish musculature . .	34
6.	Fish carcass cross-section and myocommata . . .	35
7.	Chemical death survival curves for <i>L. monocytogenes</i> , TSB and fillets acidulated to 2% lactic acid.	74
8.	Chemical death survival curves for <i>L.monocytogenes</i> , TSB and fillets acidulated to 2% acetic acid.	75
9.	Chemical death survival curves for <i>L. monocytogenes</i> , TSB and fillets treated with 50ppm sodium hypochlorite.	76
10.	Chemical death survival curves for <i>L. monocytogenes</i> , TSB and fillets treated with 1% hydrogen peroxide.	77
11.	Chemical death survival curves for mixed flora of fillets for chemical treatments. . .	78
12.	pH of catfish fillets stored over 12 days on ice after treatment (HAc and LAc at 65°C, 30 sec.).	89
13.	pH of catfish fillets stored over time on ice after treatment (HP combined with HAc and LAc 65°C, 30 sec.).	89

14.	Mean APC counts of catfish fillets stored over time on ice after treatment (HAc and LAc 65°C, 30 sec.)	90
15.	Mean APC counts during storage utilizing hydrogen peroxide and organic acids in combination (65°C, 30 sec.)	91
16.	Mean McBride counts for <i>Listeria monocytogenes</i> inoculated fillets during storage on ice after treatment (LAc and HAc 65°C, 30 sec.)	93
17.	Mean McBride counts for <i>L. monocytogenes</i> inoculated fillets stored on ice after treatment utilizing hydrogen peroxide and organic acids in combination (HAc and Lac with HP 65°C, 30 sec.)	94
18.	Abbreviated sensory instrument	101
19.	All possible combinations in triangle testing.	102
20.	Panelist ability to detect differences in cooked fillets (smell and taste).	103
21.	Panelist acceptability in triangle test of raw and treated (visual and smell)	105
22.	Electron micrograph of sanitized stainless steel	113
23.	Electron micrograph of stainless steel with <i>Listeria monocytogenes</i> biofilm	113
24.	Electron micrograph of sanitized polyethylene fillet board	114
25.	Electron micrograph of polyethylene fillet board with <i>Listeria monocytogenes</i> biofilm	114
26.	Electron micrograph of sanitized polypropylene knife handle	115

27. Electron micrograph of polypropylene knife
handle with *Listeria monocytogenes* biofilm . . 115

ABSTRACT

Listeria monocytogenes has the potential to be a serious pathogen for the compromised health segment of our population (immunocompromised, young, elderly and the pregnant). While the infectious dose is generally believed to be high 1.0×10^6 , infectious dosages of 1.0×10^3 have been reported.

Listeria monocytogenes poses a threat because of several factors: ubiquity in nature, isolation from fresh / salt waters, soil, ruminates and 17% of the human population. *Listeria monocytogenes* can to surfaces within 20 minutes, evade sanitizing, and is capable of growth from 37°C to below refrigeration temperature.

Fish and seafoods are high in water activity and undergo minimal if any processing. Most fish, mollusk, crustacea and shellfish still undergo hand filleting, picking or shucking. *Listeria monocytogenes* has been isolated from blue crab processing plants, catfish viscera and 61% of frozen seafoods and in one survey 17% of food worker's hands. While widely found in nature, *L. monocytogenes* can colonize stainless steel,

polypropylene, rubber, glass and iron surfaces and could serve as a contamination source.

These factors could coincide under the right circumstances and cause an estuarine related listeriosis outbreak. The need for a simple process utilizing GRAS (Generally Recognized As Safe) substances to suppress *L. monocytogenes* proliferation needs to be established.

Listeria monocytogenes in planktonic cultures and incubated catfish fillets were exposed to chemical dips. Linear relationships and chemical D-values for the reduction of *L. monocytogenes* in planktonic and dynamic systems were obtained. Hydrogen peroxide eliminated *L. monocytogenes* at low concentrations. Unacidulated sodium hypochlorite was not effective in reducing microbial populations in any system. Acetic, lactic acids and hydrogen peroxide lowered log counts by a minimum of 1.5.

Acetic acid (2% at 65°C dipped 30 seconds) was found to reduce natural flora and artificially incubated *L. monocytogenes* fillets up to twelve days.

Treated fillets were found acceptable to a consumer panel.

Listeria monocytogenes was found to adhere in a blood biofilm to common processing surfaces.

Sanitizing surfaces with acetic acid (70°C, 5% for 5 minutes) was effective in nearly sterilizing cutlery grade 4000 series stainless steel, polyethylene knife handles and polypropylene filleting boards.

CHAPTER 1

INTRODUCTION

The expanding world population and consumer demand for fish, coupled with decreasing world / U.S. fish catch and subsequent marine species depletion, will lead to a continued increasing reliance on aquaculture. In 1974, aquaculture provided 7% of the fish consumed; in 1984 16%, in 1994, 36% (USDA, 1995). Fish produced by aquaculture is projected to be 44% by the year 2,000 (N.A.S., 1994). Mass production of catfish will continue to increase by aquacultural practices utilizing stocked pond technology. Fresh catfish is filling an increasingly lucrative market niche in gourmet and home cooking (Israel, et al., 1993; Dellenbarger, et al., 1988).

Catfish is a firm, sweet, low-fat flesh that is continuing to grow in consumer acceptability. Catfish are a hardy, rapidly maturing fish indigenous to nearly every part of the world. Their high tolerance to poor water quality, feed diversity, disease, salinity and high stocking rates mark catfish as an ideal candidate for cultivation (Shepard and Bromage, 1988). Catfish

is the most successfully aquacultured species in the United States. Catfish production could be a vital factor to augment decreasing wild fresh and salt water landings.

Listeria monocytogenes is a pathogen that has been identified as being responsible for several food poisoning and deaths since the mid 1980's (Farber, 1991). In the latter half of the 1980's, three major Listeriosis outbreaks occurred:

1. 41 cases with 18 fatalities due to contaminated cole slaw
2. 103 cases with 40 fatalities due to contaminated cheese
3. 31 cases with 14 fatalities due to contaminated salami (Bruce and Broome, 1989).

Although never definitely linked, *L. monocytogenes* was suspected to cause a death associated with ice cream (Anonymous, 1984). *Listeria* is capable of thriving at cooler temperatures (Sorrells, 1989). Fish, molluscan and crustacea species kept on ice could serve as a reservoir for a *Listeria* outbreak.

Weagant et al., (1988) performed a bench mark study to survey the prevalence of *Listeria* in seafoods. The study showed that 61% of the seafood sampled exhibited *Listeria* species.

Therefore, there is a need to establish acceptable pasteurization methods for catfish fillets. Organic acid acidulation, hydrogen peroxide or chlorination treatments of fillets and/or water are suggested methods to reduce innate microbial flora and contamination during post harvest processing. Combining these chemical methods with 'cool' pasteurization, could extend the shelf life of fillets on ice. On a world wide basis, estimates are that 45-60% of our refrigerated foods spoil before consumption (FAO, 1994).

The use of organic acids, lactic or acetic, as an antimicrobial dip is desirable because of their effectiveness, consumer acceptance and GRAS (Generally Recognized As Safe) status (CFR 184.1061, and CFR 184.1005).

Hydrogen peroxide decomposes into molecular oxygen and water by catalase and high temperatures. Peroxide

itself is a strong oxidizer and can be effectively used to reduce microbial populations. While relegated to limited food usages in the United States, hydrogen peroxide is widely used in Europe (SOLVAY, 1960).

At high concentrations, chlorine compounds are excellent sanitizers. Even at low concentrations, these compounds are capable of retarding microbial growth. Sodium hypochlorite, as an antimicrobial dip, is used in nearly all food industries.

The combination of short time fillet immersion into hot chemical dips (dips above 65°C) greatly decrease microbial load and is detrimental to *Listeria monocytogenes* (Lovett et al., 1989). Hot dip treatment on catfish fillets in conjunction with consumer acceptance as an extension of shelf life has yet to be established. Therefore the objectives of these studies are as follows:

1. Establish death rate curves for *Listeria monocytogenes* utilizing 2% acetic acid, 2% lactic acid, 1% hydrogen peroxide and 50ppm sodium hypochlorite at 1.0×10^3 and 1.0×10^6 colony forming units per milliliter (CFU/ml)

concentrations in tryptic soy broth (TSB) (Difco, 1995).

2. Compare death rate curves for *Listeria monocytogenes* (1.0×10^3 CFU/ml) inoculated onto catfish fillets utilizing 2% acetic acid, 2% lactic acid, 1% hydrogen peroxide and 50ppm sodium hypochlorite at 1.0×10^3 (CFU/ml) with *L. monocytogenes* being recovered on McBride Agar supplemented with 5% sheep red blood cells and 1% cycloheximide (Difco, 1995).
3. Establish the shelf life of catfish fillets treated with the experimentally determined most effective bacteriocidal treatment in conjunction with a high temperature short time (HTST) pasteurization dip.
4. Establish the lethality of the determined most effective bacteriocidal dip in conjunction with HTST on the pathogen *Listeria monocytogenes* over time.
5. Establish the sensory acceptability by, a consumer panel, for fresh fillets chemically treated with the HTST pasteurization dip.

6. Establish data in regard to a 10% acetic acid solution at 65°C as a sanitizing agent for cutlery stainless steel, polypropylene knife handles and polyethylene fillet boards.

CHAPTER 2

REVIEW OF LITERATURE

History of man & food

In man's beginnings, survival depended on his ability as a hunter gatherer to garner an adequate food supply during nomadic movements. Man's first abundant food hoards occurred during mass seasonal herd and fish migrations. The knowledge to cultivate grains and harvest them in mass allowed mankind to cease following migratory game and establish villages (Durant, 1954).

Exposure to food excesses would have been useless if man had not developed the ability to preserve excess meats, vegetables and fruits for later consumption. The ability to extend food supplies year round was an essential stepping stone in the development of human civilization (Frazier and Westhoff, 1988).

Documented food gathering and preservation methods date back approximately ten thousand years. Pots used for boiling have been excavated in the Near East dating back to 8,000 B.C. The Babylonians, in 7,000 B.C., fermented beer and constructed pottery for grain storage. Pottery of this type has been excavated from

burial sites dating back to 6,000 B.C. The Sammarians, in 3,000 B.C., salted meat and fish while the Egyptians were capable of making cheese. The Children of Israel, in 1,200 B.C., salted fish from the Red Sea and fresh meat was preserved by the Romans as early as 1,000 B.C. by packing commodities in snow (Jay, 1992).

Historical appreciation & development of preservation

Though early civilizations developed the capability of preserving foods, the mechanisms by which the foods were preserved were not fully understood until approximately one hundred fifty years ago. Prior to Pasteur's studies in 1860, several wide ranging ideas explaining food spoilage were held (Stewart and Amerine, 1973).

During the Middle Ages, the belief of Spontaneous Generation, life springing from nonlife, came into existence and persisted for centuries. In 1658, Kircher, a monk, was the first to record an alternate theory to that of Spontaneous Generation. Kircher suggested that worms invisible to the naked eye were responsible for food spoilage. Without the availability of a magnifying device, his postulation

was unprovable. Von Leeuwenhoek did see microscopic life, 'wee beasties', in 1683, but no connection to food spoilage was established.

In 1765, Spallanzani, in an attempt to disprove Spontaneous Generation, demonstrated that beef broth could be preserved or 'kept' by boiling and sealing the broth from the atmosphere. However, his work was discounted because 'fresh' air, believed to be a necessary component to life, was not able to come into contact with the food substance (Jay, 1992).

Preservation took a giant step forward in 1809, when Nicholas Appert, in an attempt to win Napoleon's prize money for feeding his troops, first preserved food in champagne. He later stuffed food into the champagne bottles and boiled them rather than pickling them in alcohol; thus, canning made its debut (Bishop, 1978). However, the theory of Spontaneous Generation still persisted.

Pasteur, in 1837, finally disproved the theory of Spontaneous Generation with his Swan Neck Flask experiment. In 1860, pasteurization of wine and beer was successfully achieved and Pasteur published

Etude sur le Vin, relating the spoilage of wine to specific microbes and established their destruction by heat (White, 1976).

As a result of the Industrial Revolution and increasing population expansion, methods of food preservation on a large scale became necessary. In 1865, artificial freezing of fish on a commercial scale was accomplished. In 1878, the first successful cargo shipment of frozen beef from Australia to England occurred. In 1917, Clarence Birdseye began work on freezing foods for domestic retail trade in the United States (Farrier, 1979).

Population & seafood demand increasage

During the Industrial Revolution, the increasing food demand was directly related to what has become and will continue to be a global problem - global uncontrolled population growth and the resultant increase in food demand. Figure 1 shows the constant increase in world population since 1961 (FAO, 1994).

Consumption of fish and seafood has increased worldwide proportionally with increased population growth. In the United States, seafood and fish

consumption has risen to fifteen pounds per person per year. As a result, fish catches have increased to meet this demand (USDA, 1995). This increasing demand for seafood may yield serious implications for marine eco-webs in the future.

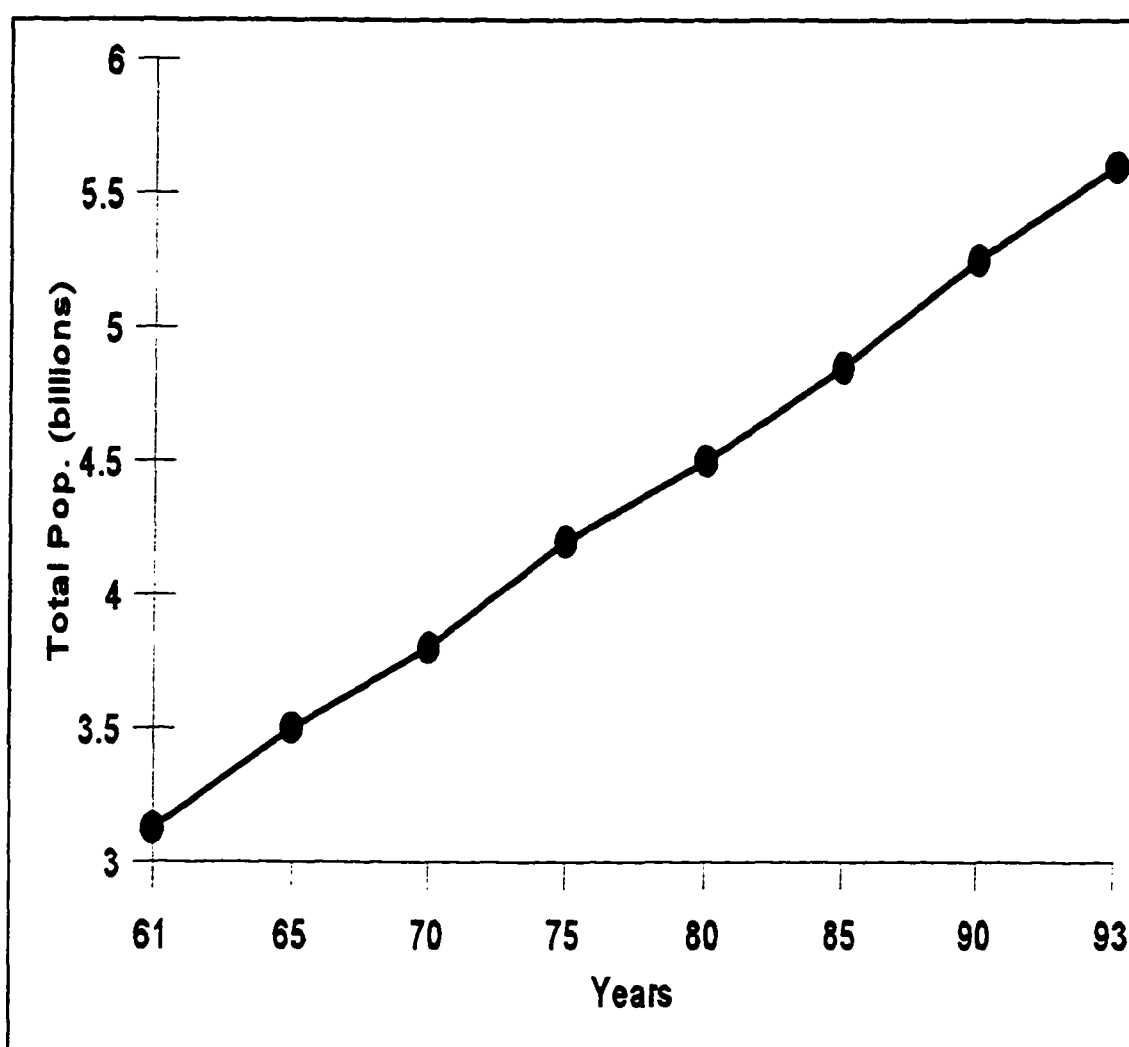


Figure 1. World population growth curve 1961-1993.
(FAO, 1994)

Fish population decline & eco-pyramid depletion

Fish population studies of yearly fish hauls suggest that the United States and the world may have over harvested one of our largest sources of protein. Figures 2 and 3 show the United States and world total marine catch since 1961. Since 1990, there has been a steady decline in both U.S. and world harvests (FAO, 1994).

Many of these harvests have been in traditional culinary favored fin fish. These species are higher order consumers in the marine eco-pyramid. Populations of these species have been depleted from habitat pollution, uncontrolled and indiscriminate harvests (Miller, 1993). Purse seines and miles of drift nets have been the primary tools responsible in indiscriminate harvest (Brown, 1988). Activist have termed drift nets 'curtains of death' and populist pressure has caused commercial drift nets to be banned in many countries. Enough commercial drift net exists to encircle the Earth twice over (Miller, 1993).

Traditional fishing methods have drastically improved hauls. Modern marine fishing fleets are

capable of staying at sea for months and have the capability of processing fish on board. Sonar and plane searches, many believe, have given the modern day fishermen an unfair advantage (Brown and Young, 1990).

Some examples of species possibly being over fished are the whitefish, haddock, flounder, cod and Pacific salmon. The cod population off the coast of Canada is so seriously depleted that the Canadian Government Fisheries Department has moved decisively to preserve this natural resource. The stringent fishing regulations and restricted number of harvesting days have led to the equivalent of a nearly true ban. The Pacific salmon runs have been increasingly poor, partially due to spawning habitat destruction. Salmon fry are being raised commercially and released into the wild to elevate fry and smelt numbers to historic levels.

As our major species (higher order consumers) are being over fished and depleted, the fishing industry is harvesting what has been argued as under utilized or 'trash fish' (middle and lower order consumers). These fish are lower on the eco-pyramid, thus have larger

populations and are being successfully introduced into our diet. As we harvest further and further down the marine food web, the more excluded the higher order fish are from their natural prey. As a result, the top order consumer fish are further hampered in their chances for species revivication (Hendry,1988).

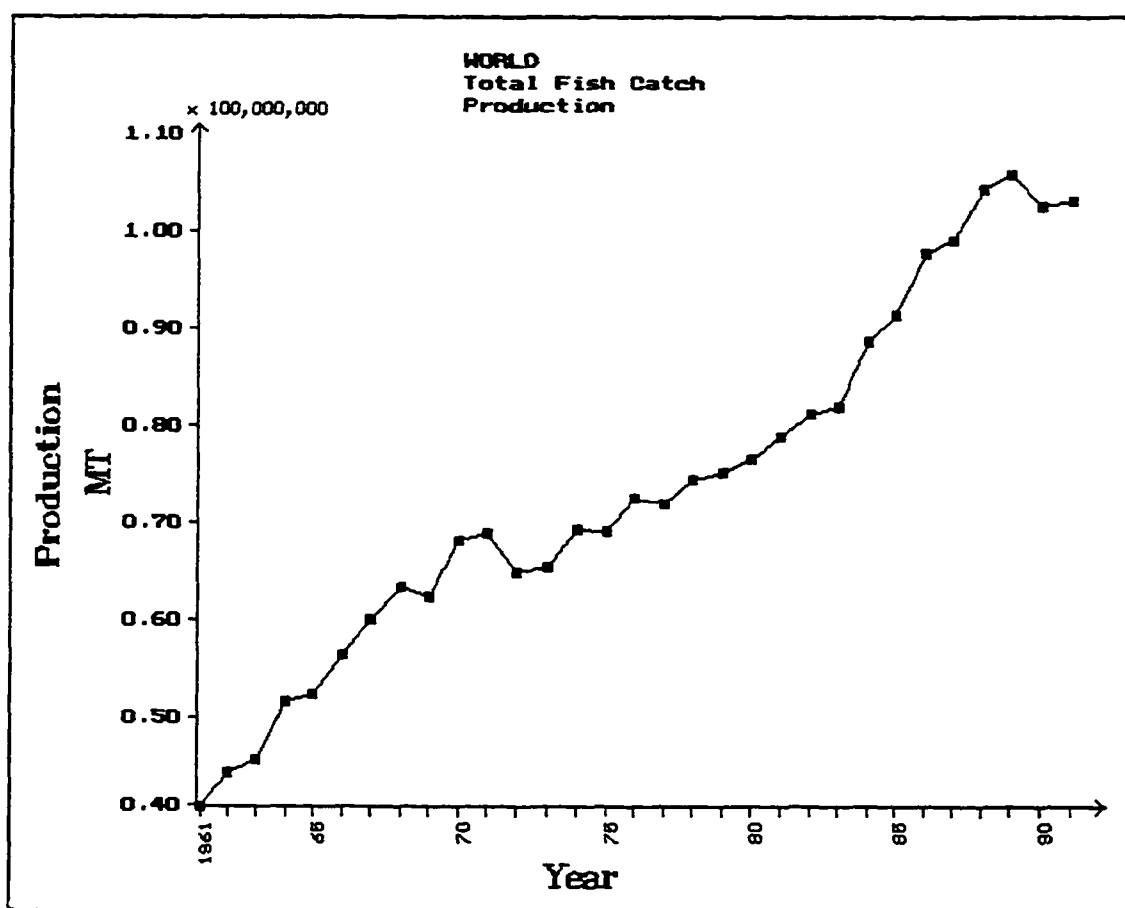


Figure 2. World fish catch 1961-1994.
(FAO, 1994)

Aquaculture history

Aquaculture is an applied agricultural science that has been practiced for thousands of years. Of the 20,000 species of fish that are known to exist, only 100 species are actively farmed and/or harvested. Worldwide, fifteen plants and eight animals make up 90% of the total food consumed (Miller 1993).

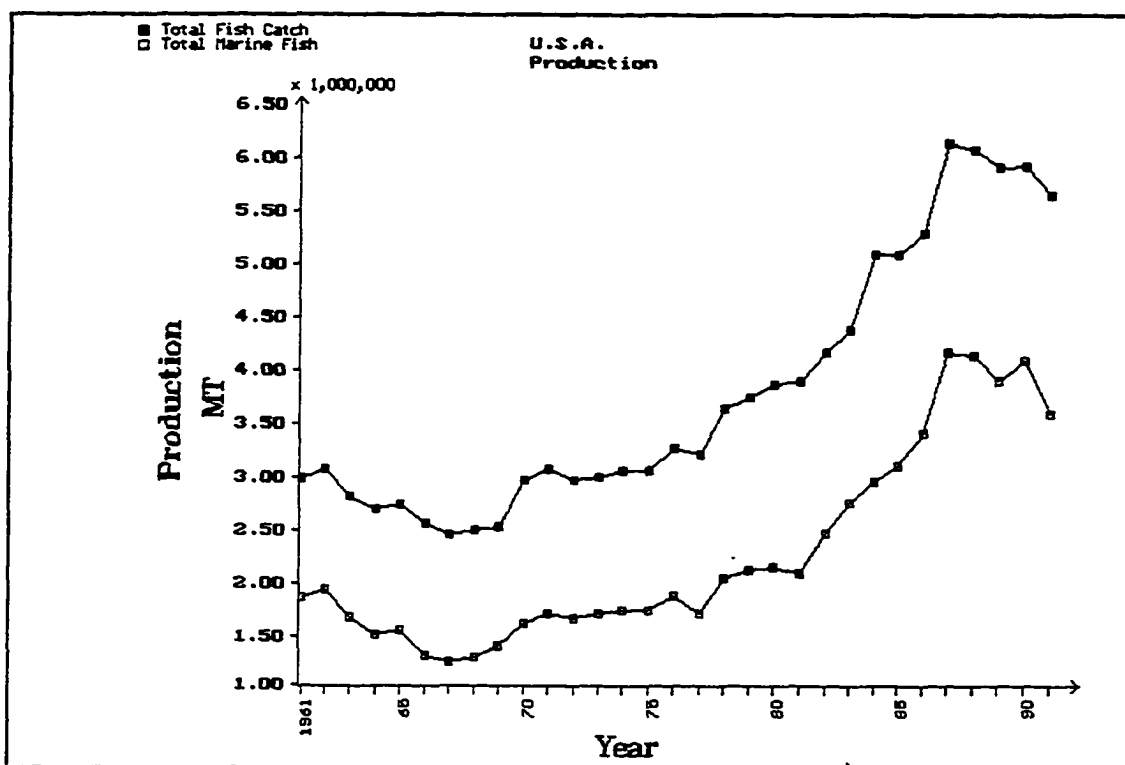


Figure 3. U.S. fish & marine fish catch 1961-1994.
(FAO, 1994)

The Chinese were known to have cultured fish as far back as 2,000 B.C. The first textbook on the practices

of raising fish, A Treatise on Carp Culture, was written in 475 B.C. by Fan Lai (Norman, 1963). Egyptian hieroglyphics of fish being raised in ponds have been found in the tombs of the Pharaohs. The Romans are credited with raising marine fish in shallow lagoons along the coast, a practice still in use today (Brown, 1988). During the Middle Ages, carp were added to moats to remove vegetation and provide a constant supply of fresh fish (Shepard and Bromage, 1988). Today, aquaculture of fish and shell fish occur in ponds, raceways, rafts, floating cages, enclosed inlets and artificial tanks (Brown, 1988).

Aquaculture today

Fish farming has both advantages and disadvantages that are often interlinked. In the United States, rearing units can range from tanks holding thousands of gallons to outdoor ponds measuring acres.

Culturing fish in a confined area allows increased control during the production cycle. Food type, consumption rate, medication, oxygenation and other monitored parameters allow substantially higher stocking rates to exist than could exist in natural

eco-systems. The result of aquaculture practice is a maximum yield of marketable fish in little space compared to that of lakes and oceans (Bilio et al., 1986).

The micromanagement of large numbers of fish insures uniform product size and high quality. Controlling the time of harvest assures the freshest product possible when demand is highest. Fish farming also eases the demand on wild stocks (N.A.S., 1994).

Rearing fish has advantages over other live stock. Being cold blooded, fish do not have to expend energy to maintain body temperature or to support weight as terrestrial animals (Shell, 1981). Fish are, therefore, inherently more efficient at converting food into flesh. Fish, in general, enjoy a 1.5 to 1 pound feed to flesh conversion ratio. When compared to other commonly eaten species (poultry 3:1, swine 8:1, and beef 18:1), fish are much more economically produced (Shepard and Bromage, 1988).

Rearing catfish in the South, however, is still as much art as a science. The intense stocking rates needed to make a profit creates a great number of

problems. The primary problem from ultra dense fish rearing is fish waste. As nitrogenous waste decomposes, ammonia develops which is toxic to fish in low concentrations. Denitrifying bacteria biologically transmute ammonia to nitrite, then to nitrate and finally to nitrogen gas (Trussell, 1972).

As fish populations become more dense, a proportional increase in bacteria occurs in water, sediment and natural skin flora , many of which are pathogens to both man and fish (Leung et al., 1995).

As harvesting innovations have helped to decimate native fish populations, emerging technologies may hold the key to feeding the world's expanding population. New advances in bioengineering could drastically alter the shape of our fishing industry. A transgenic salmon has been produced that grows several times the rate of normal salmon stocks. LSU has developed a transgenic catfish that when stressed, releases a bacteriocidal chemical that increases its ability to resist infections (Bradshaw, 1995). As a result of these factors, aquaculture is a vital endeavor to prevent the declining supplies of wild stocks, insuring a

predictable supply, quality, size, flavor and product appearance.

Catfish an aquacultural success story

Catfish are the most aquacultured fish in the Southern United States. Production of catfish in 1994, was 445 million pounds with farm revenues reaching a record high of 340 million dollars. Wild harvest of catfish could not have possibly supplied enough fish to support the requirements of today's market (USDA, 1995).

The trend in catfish farm pond acreage is increasing, with catfish farms evolving into larger and larger operations. N.A.S. (National Academy of Science) 1994, stated that in the southeastern United States there were 153,640 acres of catfish ponds in production as of 1994. An additional 9,000 acres of ponds are being rebuilt or are under construction. In 1994, the average catfish operation in Mississippi had grown to 343 acres and the average size in Louisiana increased from 64 acres in 1991, to 100 acres in 1994 (USDA, 1995).

General fish anatomy & flora

Oxygen concentration in water is one twentieth of atmospheric oxygen (dependant on altitude and temperature); therefore, the respiratory system of fish is very efficient. The ability to extract the dissolved oxygen lies in the gill structure. The surface area of gills can be 500-1000 mm² per gram of body weight for an active fish. Gill filaments are capable of extracting 60% of available oxygen. As an evolutionary result, fish hemoglobin has become more efficient at oxygen extraction than mammalian respiratory tissue (Shepard and Bromage, 1988).

Because of the rich mix of oxygen and hemoglobin, microbial counts are high in the gill area. Excessive microbial growth in the gill area is indicated by 'fishy' to putrid odors and gill appearance being dark red to deep brown. Sensory sights and smells are the first indicators of time/temperature abuse. Microbial counts are initially high in marine species due to the aqueous medium in which it lives. After harvest, bacteria must not be given the opportunity to proliferate.

Human pathogens have been isolated as part of a fish's normal flora (Leung et al., 1995). Byran (1978) found *Vibrio parahaemolyticus*, *V. cholerae*, *Clostridium botulinum* types B and E, and *Salmonella typhi* endemic to warm estuarine and marine waters. In a year long monitoring study of Seattle retail markets, Abeyta (1983), isolated *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Bacillus cereus*, *V. parahaemolyticus*, *Salmonella* and *Shigella* spp. Some of these pathogenic microbes were established to be worker transmitted. *Aeromonas* and *Pseudomonas* are common pathogens in fish and can be an opportunistic pathogen in man.

A fish's outer integument is composed of an external and internal dermis. Fish may have microbial concentrations of 10^4 - 10^6 CFU/cm² on the outer surface (Leung et al., 1995). The internal dermis is highly concentrated with goblet cells that produce the mucoid slime layer found on the exterior of fish. Fish slime provides an antibacterial barrier and water resistance (Shepard and Bromage, 1988).

Youssef et al. (1992), determined that 11.8% of microbes isolated from fish intestine were pathogenic to man. Table 1 lists the pathogens isolated from fresh water fish. Table 2 lists the pathogens isolated from catfish pond water, sediment and skin flora (Leung et al., 1995).

Of all the problems associated with aquaculture, lack of aeration is the most deadly and technologically difficult problem to date to conquer. Figure 4 shows a schematic of solid and nitrogenous waste interaction in any aquacultural practice.

Table 1. Pathogens isolated from fresh water fish.

Pathogenic Bacteria			% Abundance
<i>Proteus</i> spp.			43.5
<i>Escherichia coli</i> atypable			13.8
<i>Micrococcus</i> spp.			11.8
<i>Providencia</i> spp.			09.9
<i>E. coli</i> enterovasive			07.9
<i>Salmonella</i> spp. (4 strains)			03.9
2 wangata	1 typhimurim	1 newport	

(Youssef et al., 1992)

Nitrogenous wastes are degraded by microbial action to nontoxic substances. The ammonium ion and

ammonia, both ionized and nonionized (NH_4^+ , NH_3) are highly toxic to fish. Ammonia is first converted into nitrite, which is toxic but not as toxic as ammonia. Subsequently, nitrite is converted to nitrate, which is relatively nontoxic. Nitrate is finally converted to nitrogen gas. This conversion is metabolically accomplished by bacteria (SRAC, #2585).

Table 2. Mean counts of potentially pathogenic bacteria in water, sediment and fish viscera from catfish ponds.

Bacteria	Water ¹	Sediments ²	Fish viscera ²
<i>A. hydrophila</i>	2.40 ^b	3.61 ^b	4.20 ^a
<i>P. aeruginosa</i>	0.52 ^b	2.19 ^b	3.19 ^a
<i>Listeria</i> spp.	NA	NA	1.99

(Leung et al., 1995)

¹ Log CFU/ml of sample

² log CFU/g wet weight of the sample

³ NA: not analyzed

^{a, b} Means in a row with the same letter are not significantly different at the 0.05 level.

During summer months, oxygen availability is a constant concern. When nocturnal temperatures rise above 30°C, during June, July and August pond aeration must be supplied. Ultra high stocking rates of fish utilize large quantities of dissolved oxygen. Some recirculating aquaculture systems are equipped with

compressed oxygen feed systems to achieve proper oxygen saturation levels (Muir, 1981).

If a power failure or aerator malfunction occurs, entire ponds, thousands of fish, can be lost in minutes. Consequentially, back up systems must be purchased, installed and maintained in good operating condition. Introduction of a pathogen or toxin into an ultra high density system can result in rapid crop loss (Bradach, 1988).

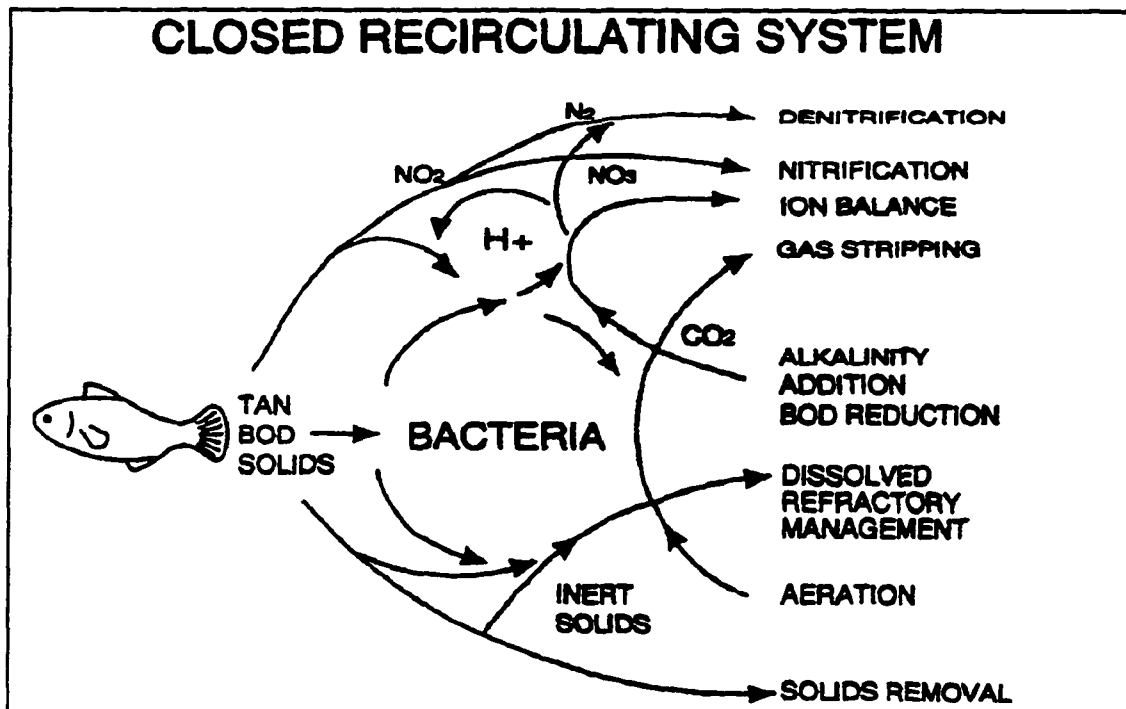


Figure 4. Nitrogenous waste bio-remediation schematic.
(SRAC, #2585)

Microorganisms

The term microorganism is a broad term ranging from protista that can be seen with the aid of a magnifying glass to *Mycoplasma* bacteria and viruses, that must be viewed with the aid of electron microscopes. The term microorganism also includes yeast and molds; however, the phrase microorganism is usually associated with bacteria. In nature, bacteria fulfill the role of pathogen, decomposer, antibiotic and food producer (Norton, 1982). Bacteria are ubiquitous in nature. There is no area on the face of the Earth that does not contain some form of prokaryotic life.

Bacteria associated with food can be divided into three major areas: pathogenic microbes, food spoilage microbes and food production microbes (Potter, 1986).

Bacteria can be classified by size, shape, flagella placement, biochemical properties and many other biochemical methods. In relation to food microbiology, the Gram stain and temperature and oxygen requirements are of paramount importance. When processing foods, the natural flora of the material is

introduced to a hostile environment eliminating or reducing the microbial population. By altering a vital requirement(s), a product's availability can be dramatically increased (Jensen, 1982).

The Gram stain was developed by a Dutch microbiologist Sir Christopher Gram in 1884. Gram established that all bacteria can be classified by a primary staining with crystal violet (purple in color) and is denoted as being Gram positive (G (+)). Some bacteria will not absorb crystal violet but do absorb the counter stain saffron (red in color) and are denoted as Gram negative (G (-)). G(+) and G(-) bacteria contain pathogenic and spoilage genera. In humans, Gram negative bacteria tend to be more common as pathogens than Gram positive bacteria.

The type of bacteria that covers a product is known as flora. The flora of a product will be a wide mixture of bacteria of both Gram stains. In nature, each animal and plant contains a unique flora that is unique and will be influenced by the native environment.

The knowledge of an organism's external, internal and environmental flora is vital when processing methods and treatments are being considered (Leung et al., 1995; Youssef et al. 1992).

Microbes & temperature

Bacteria, vegetative cells, are capable of growth in temperatures ranging from -10°C to 90°C. Bacterial groups are roughly divided into three temperature regions. Bacteria capable of living in temperatures ranging from -10°C to 20°C are termed psychrophiles, cold loving. The natural habitat for these organisms is colder waters and soils. Psychrotrophic bacteria are not normally human pathogens, with *Homo sapien* body temperature being 37°C. These bacteria are able to live and reproduce in foods at refrigerated temperatures. Therefore, food spoilage and toxin production can occur in a condition (refrigeration temperatures) that is usually considered safe (Jensen, 1982). There are two pathogen groups that are able to proliferate in refrigerated conditions. These pathogens are *Listeria spp.*, *Aeromonas spp.* and specifically *Yersinia enterocolitica*.

Most bacteria are classified as mesophilic living in a temperature range of 20°C to 50°C. Mesophyls are of great importance because the vast number of pathogens that persist are in this temperature range.

Thermophiles are bacteria capable of surviving and reproducing at temperatures ranging from 40°C to 90°C. These bacteria are usually isolated near volcanic vents, hot springs and bubbling mud pits.

Knowledge of essential living requirements of bacteria is vital to controlling their growth. When bacteria of one temperature range are moved into another, survival and reproduction is sharply curtailed, if not eliminated. This knowledge can be of great economic importance when processing and storing food (ICMSF, 1980).

Microbes & food spoilage

By definition, food spoilage occurs when a food is rendered aesthetically unusable or unfit for consumption by man or animal. Spoilage occurs from the breakdown of lipid, carbohydrate or protein components of a food. Innate proteolytic/lipolytic enzymes or bacterial metabolism of nutrient sources result in

several chemical and enzymatic by-products. These by-products may be further reduced, oxidized or metabolized to render the food stuff sensorially unacceptable. Many final or intermediate metabolites are detectable by the human senses. Human perception cannot serve as the only indicator of wholesomeness. Bacteria may exist, reproduce, excrete enzymes, toxins and chemicals that are not detectable by the human senses, but nevertheless can cause sickness or death (ICMSF, 1986).

Microbial adhesion

The formation of a microscopic layer of microorganisms onto a physical surface is commonly known as biofouling or biofilm development. Biofilms will eventually cover any structure that is in contact with a nutrient media (Criado et al., 1994). Bacteria have been shown to adhere to inorganic surfaces such as rubber & Teflon®, stainless steel/glass/& polyethylene and cast iron (Mosteller and Bishop, 1992; Mafu et al., 1990; Spurlock and Zottola, 1991) as well as tissue layers in a living host (Shand et al., 1985). In a living host, bacterial adhesion may exist as normal

colonizing flora or may adhere prior to pathogenesis. In food processing plants, the adhesion of bacteria to contact surfaces could potentially cause sensory, economic and health problems (Videla, 1989).

Microbial adhesion was first studied by Zobell (1943). Bacterial attachment to glass slides in aquatic and soil environments were established. Regardless of environment, microorganisms attach and initiate growth on a surface (substrata) by long polysaccharide fimbriae or glycocalyx. Glycocalyx form a mat or a continuance tangled covering referred to as felt. The polysaccharide strands secreted by bacterial and prokaryotic cells vary and may be responsible for tissue specificity (Shand et al., 1985). In eukaryotic systems, the type of surface, availability of divalent cations, media, bacterial genera and even species serotype may help dictate the polysaccharide produced. Several genes may code for different glycocalyx. The bacteria may shuffle through the genes producing several polysaccharide chains, evaluating each tendril's ability to adhere to the surface in question. The method by which the bacteria evaluates the

differing polysaccharide strands for attachment is not known (Costerton et al., 1978).

In planktonic cultures however, bacteria do not expend energy to maintain a glycocalyx felt. The energy for attachment is not needed when nutrients are readily available (Costerton et al., 1978).

Two basic theories of bacterial attachment have been advanced. In the most basic theory, the bacteria makes random contact with a solid surface, which establishes weak and reversible associations between the microorganism and substrata continually initiating and breaking contact. After a time, more glycocalyx are produced, thickened and irreversible attachment is advanced thereby drawing the bacteria closer to the surface (Busscher and Weerkamp, 1987).

Biofilms may form in a 5 stage process involving physical, chemical and biological factors.

1. Transport of nutrient to the solid surface
2. Adsorbtion of a conditioning film
3. Attachement of microbial cells to a wetted surface and initiation of growth
4. Bacterial metabolism within the biofilm

5. Cell disruption, growth and detachment
from the biofilm (Characklis, 1984).

The fact that bacteria are able to form adhesions is not in question. *Listeria monocytogenes* has been specifically linked to differing substrata, meat surfaces, Dickson (1990) and Chung et al. (1989), and food service worker's hands (Kerr et al., 1993). This undetachable source of a pathogenic bacteria poses a potential health problem.

Microbes & pH

The unit of pH is a measure of the degree of acidity or basicity. By controlling the degree of acidity or basicity, microbial growth can be controlled. Microorganisms, in general, grow best at pH values in the range of 6.6 to 7.5. Few bacteria grow below 4.0 or above 10.0 (Potter, 1986).

The pH is a measure of the concentration of the molar hydrogen ion concentration in solution. As the external environment of the bacteria is concentrated with aqueous hydrogen ions, the cell membrane begins to lose integrity and the membrane becomes increasingly permeable. As the extracellular

hydrogen ions flow into the cell, due to the concentration gradient differential, the interior pH of the cell begins to decline (Davidson and Branen, 1993). The conformational shapes of cellular enzymes and nucleic acids are pH dependant. As the hydrogen ion concentration increases, the isoelectric point of cellular constituents may be shifted, resulting in new chemical bonds formed with a rearrangement of the tertiary and quaternary protein structures. As a result, cells die or become incapable of reproducing. The most important food pathogen, *Clostridium botulinum*, cannot grow below a pH of 4.6 (Jay, 1992).

Fish protein

Fish muscle is composed of a series of wave shaped muscle blocks arranged along the length of the body. These blocks alternately contract on each side allowing a flex or S-shaped motion to provide propulsion. Figure 8 shows a dorsal and lateral view of fish musculature (Shepard and Bromage, 1988). Each block of fish muscle is connected by thin sheets of collagen. The unit between two sheets of collagen is termed a myocomatta which is illustrated in

Figure 9. This arrangement is commonly observed by 'flaking' of fish flesh after cooking. The flaking is the muscle block being freed from the perimysium. In a reversal from mammals, older fish have a lower

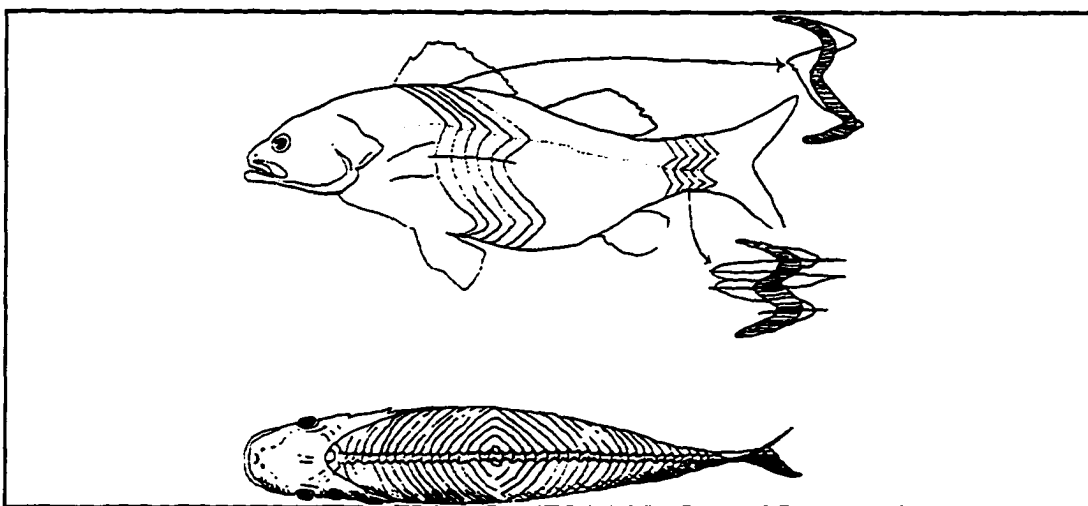


Figure 5. Lateral and dorsal view of fish musculature. (Fennema, 1985)

percentage of crosslinking and the crosslinking is not as strong as in young fish (Fennema, 1985). Fish do not have high levels of collagen and are quickly degraded by heat and enzymes (Webb and Howell, 1975). Cod contains 3%, sole 4%, and dogfish 10% connective tissue (Buttkus and Tomlinson, 1966).

Kenji et al., (1993) found type V collagen in trout was degraded during chilled storage (1 day at

5° C) resulting in a significant decrease in textural firmness in sensory and sheer test. Alkaline protease activity in mullet was found active at pH 8 and had significant textural changes measured by

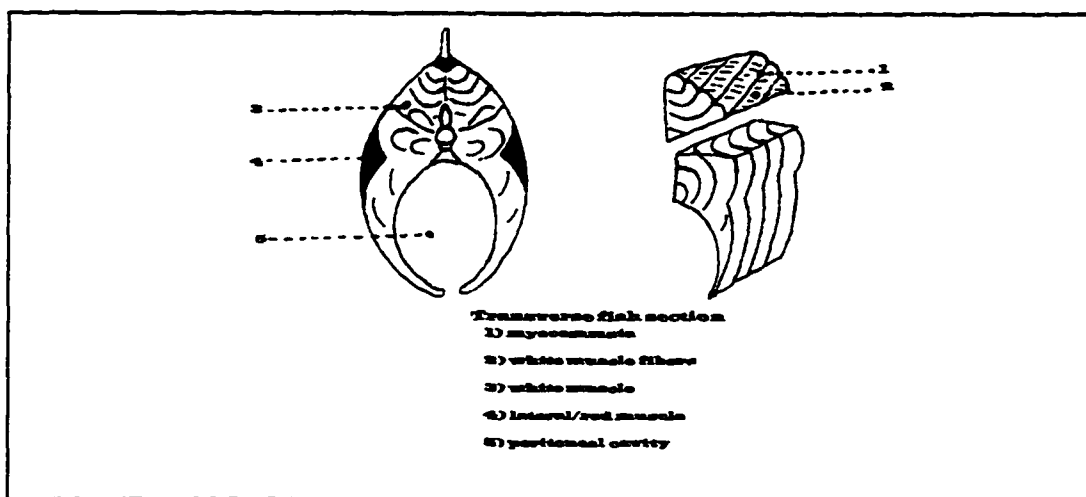


Figure 6. Fish carcass cross-section and myocommata.
 (Fox and Condon, 1985)

Kramer sheer force measurements (Deng, 1981).

As resolution proceeds, an increasing amount of myofibular fragmentation occurs, which is associated with tenderness. The high concentration of calcium ions, activation of proteases, release of lysosomal enzymes and increased lactic acid are all contributing factors to muscle resolution.

The final pH of muscle can be an important factor in both muscle toughening and tenderization.

Final pH of muscle is primarily due to conversion of glycogen to lactic acid occurring under anaerobic condition. The pH in warm blooded muscle may drop to a pH of 5.5. A rapid drop or final low pH may result in denaturation of sarcomeric proteins and increase in toughness and dryness. As the concentration of sarcoplasmic proteins decrease, so does the water holding capacity of the muscle. Feinstein and Burke (1972) found a similar relationship of declining pH and increasing muscle toughness in both flounder and cusk.

The water holding capacity and juiciness are closely related. If the final pH is high, the sarcoplasmic proteins are far from their isoelectric point and are able to bind more water. At high pH's, meat may appear puffy and exudative (Honikel et al., 1981).

The post rigor pH of fish (approx 6.5) is much higher than that of hooved animals or fowl. If the pH decreases into the 5.5 to 6.5 range, the myotomes in fish may weaken or break away from the myocommata and cross linking of the myofibular complex

(toughening) occurs. If the fish is further processed or frozen, the damage to the flesh in addition to the weakened myocommata structure, may yield an extremely poor quality and unacceptable fillet. The myotomes may break apart in a phenomenon known as gaping (Fox and Condon, 1985).

Sensory analysis

Sensory analysis is a method of determining food quality by the consumer. Despite many advances in food science, if the consumer deems the product sensorally unacceptable, the usefulness of the product or treatment becomes academic.

Sensory panelists may be trained or untrained to determine color, appearance, odor of cooked/raw product, flavor and mouth feel. The subjective scores of a trained panelist can be considered objective (Trant et al., 1981; Pangborn, 1989).

Sensory panelist scores are often correlated to laboratory tests to establish mechanical indicators of acceptability. Rossler et al.(1956) found that odor was the simplest and most accurate measurement of freshness in fillets and headless European hake.

Hollingworth et al. (1991) found valid comparisons between sensory scores and microbial/chemical indicators in pasteurized, vacuum packed surimi crab meat. Loung et al. (1992) used sensory panelists to measure texture, appearance, and odor of raw and cooked fish and could not correlate consumer acceptability to nucleotide degradation.

Finally however, sensory testing is concerned with measuring physical properties by psychological techniques. In psychometrics, sensory methods are used for measurements that cannot be made directly by physical or chemical tests (ASTM, 1993), although correlation may occur.

Each of the five senses has its own unique receptors and neural tracts to higher order complex brain centers (Pfaffman et al., 1954). As sensorial information is transmitted to perception centers, signal integration occurs and acceptability or rejection is determined. (Stone and Pangborn, 1968).

Discrimination testing

Discrimination testing is a useful analytical tool to test perceived differences between two

products or processes. If differences are perceived, justification for descriptive tests to determine magnitude and direction may be warranted as the next step in process or product evaluation.

Triangle testing is designed to perceive a difference in a treatment and a control. Triangle testing is performed by presenting a series of three choices. Some of the choices contain no stimulus (Dember, 1963). The panelist is to indicate whether or not the stimulus is present in the three choices. Panelist are not required to identify the stimulus, only indicate perception. If the treatment is identified, recognition and directional tests may be employed to establish the acceptable maximum and minimum tolerances (Woodward and Schucany, 1977).

Electron microscope

The electron microscope has the ability to detect and show bacteria adhering to surfaces. Bacterial adhesion to food processing surfaces are potentially health hazards.

The electron microscope uses accelerated electrons that have a wavelength that is only a

fraction of the wavelength of light. This short wavelength greatly increases the resolution and discrimination of minute structures. There are several types of electron microscopes available today for use. In microbiology, the transmission electron microscope (TEM) and the scanning electron microscope (SEM) are the most widely used imaging instruments.

The TEM employs an electron gun as a source of electrons. Electrons are discharged by a cathode and accelerate toward a circular anode. The electrons are emitted through an aperture in the anode at high speed, forming an electron beam. The beam, traveling within a vacuum, is directed toward the specially prepared, ultra-thin specimen. An electromagnetic condensing lens concentrates and focuses the beam. The electron beam passes through the specimen chamber where the electrons are absorbed or deflected by the specimen. The residual beam of electrons is focused and magnified by electromagnetic lenses and projected onto a fluorescent screen.

SEM utilizes the specimen that has been covered with a thin film of gold to increase electrical

conductivity and decrease blurring. The electron source emits the electron beam toward the specimen. An electromagnetic condensing lens concentrates and focuses the beam. SEM employs a scanner to permit the electron beam to sweep back and forth across the rough "hills and valleys" surface of the specimen, inducing secondary emissions of electrons from the surface. A signal detector captures the electrons and, line by line, builds an image on a monitor. Electrons that strike a sloping surface are deflected such that not all of the deflected electrons are captured by the signal detector. This generates a greater degree of contrast and produces a three-dimensional effect (Hayat, 1987).

Stainless steel

Many bacteria are capable of colonizing and attaching to nonnutritional surfaces. Stainless steel is a surface that is commonly found throughout the food industry. *Listeria spp.* has been shown to attach to stainless steel pipes and surfaces in dairy plants. Dairy plants utilize what is commonly known as 3,000 series stainless steel. Cutlery steel is

made from 4,000 series stainless steel. The adjuncts added to steel dictate the usage and the numeric denotation.

The American Iron and Steel Institute (AISI) has devised a system of classifying different types of steel. The system utilizes a 4 digit code. Common dairy pipes and counter tops are generally composed of '3000 series' stainless steel. The 3000 series steel will not sharpen or hold an edge and is unsuitable for cutlery. Cutlery steel is always a 4000 series stainless steel.

The first number in describing steel indicates the principle metal alloyed (<1%). The second number indicates the approximate percentage of the second metal (<1%) alloyed. The third and fourth numbers indicate the percent residual carbon in the steel (>1%). Fillets knives that are 4004 would contain 0.4% molybdenum and 0.4% carbon. Carbon content between 0.3 and 0.6 classify steel as mid-carbon steel which posses the general properties of low ductility, great hardness and high tensile strength. Alloyed molybdenum is added for toughness and shock

resistance. The following digits indicate the series type of steel:

1 - carbon, 2 - nickel, 3 - nickel/chrome,
4 - molybdenum, 5 - chromium, 6 - chromium/vanadium,
7 - tungsten, 8 - nickel/chromium/molybdenum,
9 - silicomanganese (Repp and McCarthy, 1989).

Chemical agents

There are a variety of chemicals that can be added to foods to retard microbial growth. Chlorine and short chain fatty acids are among the most common found in the food industry.

Chlorine

For many years the disinfection and sanitation of water, food products and food processing equipment has been based on the use of halogen based disinfectants, such as chlorine or iodine (Biocide Intl. Corp., 1994). The use of chlorine on a worldwide basis has improved the quality of life and prevented many outbreaks of waterborne transmission of both pathogens and spoilage microorganisms. Chlorine has been used in the sanitation and sterilization of food and food processing equipment

for many years (Owusu-Yaw et al., 1990). Chlorine was first used as a disinfectant in 1897, when chlorine compounds were used to disinfect polluted water mains after an outbreak of typhoid fever in England (Sconce, 1962). In 1912, Whittaker and Mohler described the use of calcium hypochlorite in the sterilization of milk bottles in the American Journal of Public Health (Whittaker and Mohler, 1912). That same year, water mains were once again flushed using chlorine after a typhoid fever outbreak, this time in Niagra Falls, New York (Sconce, 1962). Chlorine became widely used in the fishing industry in 1935, and has since become one of the most widely used disinfectants in the fishing and fish processing industries (Fitzgerald and Conway, 1937).

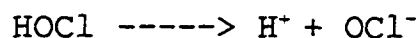
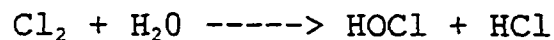
The single most important factor in the storage and shelf-life of food products is the efficacy of microbial decontamination prior to storage (Ayres et al., 1950). A number of studies have shown that a satisfactory method of extending the shelf-life of fresh fish and shellfish would be to utilize a pre-

storage method of microbial decontamination (Kosak and Toledo, 1981). The use of a bactericide in treatment waters for food products is, therefore, a very common practice in the food industry. Both chlorine and chlorine dioxide treated waters are more effective in microbial disinfection of chicken broiler carcasses than untreated water (Lillard, 1980).

Hypochlorous acid is an excellent bactericide because of its ability to diffuse through the bacterial cell wall and damage vital cell parts. This is thought to be due to the reaction between hypochlorous acid and the enzymes present in the cell (Sconce, 1962).

Chlorine and other disinfectants are lethal upon contact to a portion of bacteria. By interrupting reproductive and growth cycles, the eventual death of bacteria can be achieved (Singh et al., 1990). Chlorine acts through a mechanism of chlorination based on the process of electrophilic substitution (Biocide Intl. Corp., 1994). When chlorine gas or sodium hypochlorite is dissolved in water in a low pH

condition (pH < 5.6), the following reactions occurs:



Since the chlorine ion in hypochlorous acid has a valance of plus one, this ion is considered the 'active chlorine' component.

Industrial uses of chlorine for treatment of food products have generally been used at concentrations as high as 200-250 ppm chlorine, or 100-125 ppm concentration of available chlorine. Some studies have even indicated that the effectiveness of chlorine as a disinfectant is maintained without appreciable off odors or flavors when the concentration is as high at 1,000ppm (McVicker et al., 1958). These concentrations are of concern due to the capacity of chlorine to be mutagenic (Emswiler and Kotula, 1976). USDA is the maximum level of residual is 50ppm chlorine. However, Lopes in 1986, reported that 100 ppm available chlorine as sodium hypochlorite and dichloroisocyanurate was effective for a 99.99%

reduction of *Listeria monocytogenes* and *Salmonella typhimurium* within thirty seconds. Criado et al. (1994) reported that low chlorine levels of 0.5-5 ppm were only inhibitory when used on biofilms. El-Krest and Marth, in 1988, tested exposure of *Listeria monocytogenes* with sodium hypochlorite and concluded that a program of cleaning and sanitizing with sodium hypochlorite should be able to control nonspore forming organisms including *Listeria monocytogenes*.

Organic acids

All microbes have a tolerance to pH possessing a minimum, maximum and optimum level for growth. When those extreme levels are exceeded, either death or inactivation of the bacterium may occur (Norton, 1982). Inorganic action is based upon the dissociation of the hydrogen into the hydronium ion. In organic acids this also occurs but to a lesser degree. The pKa of an organic acid is defined as that point, pH, when 50% of the acid molecules dissociate from the organic moiety. The pKa of most organic acids is between pH 3 and 5. A list of organic acid pKa's are found in table 3.

While lowering the pH of a food may create an unfavorable environment, the number of microbes, species present, type of acidulant, concentration, time of exposure, temperature

Table 3. Dissociation constants of organic acids in aqueous solutions.

Acids	pK_1	pK_2	pK_3
Acetic	4.75	---	---
Dehydroacetic	5.27	---	---
Sodium diacetate	4.75	---	---
Adipic	4.43	5.41	---
Caprylic	4.89	---	---
Citric	3.14	4.77	6.39
Fumaric	3.03	4.44	---
Lactic	3.08	---	---
Malic	3.40	5.11	---
Propionic	4.87	---	---
Succinic	4.16	5.61	---
Tartaric	2.98	4.34	---

(Davidson & Brannen, 1993)

and buffering capacity of the food will all play a part in the microbe's ability to survive (FAO, 1994). Increasing acid concentration can certainly be a detriment to microbial growth. Undissociated organic acids also exhibits antimicrobial effects.

Undissociated organic acids exert some degree of lipolytic action. The acid chain is able to dissolve into the fluid membrane and can disrupt permeability as well as the proton gradient. When using organic acids as an acidulant many factors will dictate its efficacy; concentration, chain length and degree of branching. The choice of acidulants may also depend on other factors. The tartness of acids may or may not be appropriate. The acids may act as chelators, or as antioxidants and interfere with browning all which may or may not be favorable and product dependant.

Acetic acid

Acetic acid is a monocarboxylic acid with a pungent odor and taste. This acid is the principal component of vinegars and as such is primarily used for its flavoring abilities. Acetic acid is highly soluble in water. This acid is generally recognized as safe (GRAS) for miscellaneous and general purpose usage (CFR 21 CFR 184.1005, 1995).

Acetobacter and certain lactic acid bacteria are tolerant of acetic acid owing to their association

with acids in fermented products. Woolford (1975), confirmed the effectiveness of acetic acid at pHs 6, 5 and 4. The higher pKa allows more molecular acetic acid enter the cell, dissociate and cause cell detriment. Heterolactates were more resistant to acetic acid than homolactates. Gram negative bacteria were more inhibited than lactic acid bacteria, *Clostridium*, Gram positive bacteria, yeast and molds. As the pH was decreased to 4 the latter five were affected.

Fabian and Wadsworth (1939), showed that on an equal acidity basis, acetic acid was more inhibitory than lactic acid. In conclusion, pH was not a reliable indicator of preservation value except in *Clostridium botulinum* where a pH of 4.5 is inviolately inhibitory. Nunheimer and Fabian in 1940, used *Staphylococcus aureus* to rate the effectiveness of acids. Tartaric acid had one of the highest dissociation constants, yet exerted the weakest inhibitory action. Acetic acid was found to be the most effective acid treatment, but had the lowest dissociation constant, thus confirming that

the antimicrobial activity must be due to undissociated acid molecules.

Acetic acid caused greater inactivation of *Listeria monocytogenes* than lactic and citric acids and inhibition increased as temperature of incubation decreased (Ahamad and Marth, 1989, 1990). Sorrells et al. (1989) compared acetic, citric, malic, lactic and hydrochloric acids for antimicrobial activity against *Listeria monocytogenes*. At equal pH values, acetic acid was superior to lactic, citric, malic and hydrochloric acids at all incubation times and temperatures. The minimum inhibitory pH level was pH 4.4 for malic, citric and hydrochloric acid, pH 4.4-4.6 for lactic acid and pH 4.8-5.0 for acetic acid. Sorrells et al., (1989), demonstrated interactive effects between sodium chloride, acidulants, and temperature.

Blankenship (1981), demonstrated the interrelationship between temperature and acid concentration using *Salmonella bareilly*. The rate of injury increased with increasing acid concentration on the basis of a 90% decline (1-D value) in

population. Injury and death rates did not change at refrigeration temperatures, thus no relationship existed.

Anderson et al. (1977), reduced bacterial counts by 99.6% (~1.2-D value) on meat using a 3% concentration of acetic acid before washing. Acetic acid was a superior sanitizer when compared to hypochlorite and had a greater residual effect. Further work by Anderson et al. (1977) demonstrated that a 3% concentration of acetic acid at 70°C was most effective in sanitizing beef semitendinous muscle inoculated with *Escherichia coli*, *Salmonella typhimurium* or a manure slurry. Increased temperature was found to play a greater role in reducing microbial numbers than acid concentration.

Poultry scald water with 0.1% acetic acid held at 53°C decreased levels of *Salmonella typhimurium* and *Campylobacter jejuni* by 0.5-1.5 log CFU. Addition of 1% acetic acid caused instantaneous death of both microorganisms (Okrend et al., 1986). Levels of 0.3-0.5% acetic acid reduced *Enterobacteriaceae* counts 2.24 log (Lillard et al., 1987).

Lactic acid

Lactic acid is a hygroscopic and syrupy liquid having a moderately strong acid taste. Lactic acid is used commonly in the manufacture of jams and jellies. Lactic acid is also a common and primary agent in fermented foods. Lactic acid is approved as a GRAS substance for miscellaneous or general purpose usage (CFR 184.1061, 1995).

Lactic acid has been found to be more effective against *Bacillus coagulans* and *Mycobacterium tuberculosis* than other organic acids. Lactic acid sprays have been effective in limiting microbial growth on meat carcasses under a variety of storage condition. A 1-1.25% concentration of lactic acid sprayed on veal carcasses followed by vacuum packaging lowered microbial count after storage for 14 days at 2°C (Smulders and Woolthus, 1983). A 2% treatment at pH 2.3 of veal tongues, combined with vacuum packing and storage at 3°C reduced aerobic mesophilic plate counts from 5.6 to 2.7 log CFU/cm² (Visser et al., 1988). Sprays of 1% were effective in significantly reducing total counts on skinned cow

heads stored at 4, 15 and 20°C (Cudjo, 1988). Lactic acid was able to extend shelf life by 3 days at 4°C and 1 day at 15 and 20°C. Woolthus et al. (1984) effectively reduced total plate counts and *Enterobactaceae* and *Lactobacillaceae* counts by 2-3 log by immersing porcine livers in 0.2% lactic acid for 5 minutes.

Lactic acid dips have also been used successfully in poultry products. Total microbial numbers from broilers immersed for 15 seconds at 19°C in 1 or 2% lactic acid at pH 2.2 decreased from 5.2 to 3.7 log CFU/g (Van der Marel et al., 1988). Levels of psychotrophs decreased from 3.9 to 2.7 log CFU/g and *Enterobacteriaceae* from 3.3 to 2.6 log CFU/g. Higher concentrations of acid did not ensure greater decontamination nor did repeated treatments.

Peroxides

Peroxides are compounds that contain at least one pair of oxygen atoms bonded by a single covalent bond. Each oxygen is loosely bound in the molecule and is readily released as active oxygen; therefore, peroxides are referred to as oxidizing agents.

Peroxides exist in both organic and inorganic formula.

The most widely used peroxy compound in disinfection and preservation is hydrogen peroxide. Hydrogen peroxide as an antimicrobial agent was first discovered by Louis-Jacques Thenard in 1818. Hydrogen peroxide was soon introduced into the medical practice to reduce foul odors. Hydrogen peroxide has been primarily used as a topical disinfectant at a 3% concentration (Schumb et al., 1955).

Hydrogen peroxide is becoming more and more common in food processing areas. Hydrogen peroxide has a relatively low toxicity and decomposes into water and oxygen. Currently, hydrogen peroxide is used widely in countries other than the United States. Hydrogen peroxide has been approved in dairy manufacturing and some poultry applications, with the condition that catalase, an enzyme that decomposes peroxide, is added. The Food and Drug Administration has approved the use of hydrogen peroxide for the sterilization of equipment and containers in aseptic

packaging of foods and drink (Brock et al., 1988). Usage as an antimicrobial treatment has been allowed only in specific cases. The addition of peroxide has in the past been considered adulteration. This is not the case in other countries. SOLVAY (1960), established that peroxide does not act antimicrobially as a molecule but by the production of powerful reaction products such as singlet or superoxide oxygen. Superoxide oxygen radicals are a very high energy form of oxygen, highly reactive and detrimental to cell membranes. Superoxide radicals may be generated through the lactoperoxidase and myeloperoxidase enzyme cascade. These enzymes are in high concentration in leukocytes. In these cells there is a metabolic balance between peroxide production and catalase generation (Russell, 1990).

Superoxide is highly reactive and can cause oxidative destruction of lipids and other biochemical components. This radical is a potent oxidizing agent and is capable of attacking any of the cell's organic components, many of which could be essential to metabolism (Brock et al., 1988). Gould and Dring

(1975) theorized that the antimicrobial activity of hydrogen peroxide comes from the oxidation of sulfhydryl groups and double bonds in proteins, lipids and surface membranes. Peroxides are most effective against anaerobes. Since anaerobes cannot tolerate oxygen, these genera tend to have little or no catalase capability. Peroxides are generally more bactericidal to Gram negative bacteria than Gram positive.

Concentrations of hydrogen peroxide of 0.001-0.1% at room temperature are sufficient to inhibit the growth of bacteria and fungi. At 0.1% and higher, antifungal and bactericidal effects are seen. Hydrogen peroxide is more effective in acidic solution than neutral or basic (Baldry, 1983).

Survival curves & D-Values

A chemical death rate curve is of a similar concept as that of a thermal death rate curve. Chemical death rate curves provide data on the rate of destruction of a specific organism in a specific medium for a specific concentration of chemical. The death / concentration / time relationship is obtained

by graphing the logarithm of the surviving fraction versus time for a constant concentration.

Conditions must be defined to make a death curve meaningful and applicable to a food. Curves will be affected by initial microbial population, the type of food and exposure temperature.

The measure of the chemical death curve's effectiveness is the D-Value. The D-Value is the time required for a reduction in viable cells by 90% or 1 log of the bacterial population. D-values are calculated from the linear portion of bacterial semi-log survival plots. Using linear regression analysis, the D-Value = $-1/\text{slope}$ of the regression curve (Jackson and Shinn, 1993).

Listeria monocytogenes

General information

This is a motile Gram-positive bacterium. Some studies suggest that 1-10% of humans may be intestinal carriers of *L. monocytogenes*. *Listeria* spp. has been found in at least 37 mammalian species, both domestic and feral, as well as in at least 17 species of birds and possibly some species of fish

and shellfish. *L. monocytogenes* can be isolated from soil, silage, and other environmental sources (Gellen et al., 1987). *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heating remarkably well for a bacterium that does not form spores (Welshimer and Donke-Voet, 1971).

Listeriosis is the name of the general group of disorders caused by *L. monocytogenes*. Listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site, placenta or fetus (Busch, 1971).

The manifestations of listeriosis include meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. The onset of the aforementioned disorders is usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may precede more serious forms of listeriosis or may be the only symptoms expressed.

The incubation time for serious forms of listeriosis is unknown but may range from a few days to three weeks. The onset time for gastrointestinal symptoms is unknown but is probably greater than 12 hours (Schleech et al., 1983). The infective dose of *L. monocytogenes* is unknown but is believed to vary with the strain and susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, "it is safe to assume that in susceptible persons, fewer than 1,000 total organisms may cause disease" (Fleming et al., 1985).

Listeria monocytogenes may invade the gastrointestinal epithelium. Once the bacterium enters the host's macrophages, or polymorphonuclear cells, the bacteria is blood borne (septicemic) and can grow. The pathogen's presence, intracellularly, also permits access to the brain and probably transplacental migration to the fetus in pregnant women. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic cells (neutrophils, basophiles, acidophiles and monocytes) (McLauchlin et al., 1986).

Diagnosis of human illness

Listeriosis can only be positively diagnosed by culturing the organism from blood, cerebrospinal fluid, or stool, although the latter is difficult and of limited value (Albritton et al., 1980).

Associated foods

L. monocytogenes has been associated with such foods as raw milk, supposedly pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish. *L. monocytogenes*' ability to grow at temperatures as low as 3°C permits multiplication in refrigerated foods (Fleming et al., 1985).

Disease frequency

The 1987, incidence data collected by CDC suggests that there are at least 1600 cases of listeriosis with 415 deaths per year in the U.S. The vast majority of cases are sporadic, making epidemiological links to food very difficult.

Most healthy persons probably show no symptoms. The "complications" are the usual clinical expressions of the disease. When listeria meningitis occurs, the overall mortality may be as high as 70%; from septicemia 50%, from perinatal/neonatal infections, greater than 80%. In infections during pregnancy, the mother usually survives.

Successful treatment with ampicillin has been reported. Trimethoprim-sulfamethoxazole has been shown effective in patients allergic to penicillin (CDC, 1994).

Target populations

The main target populations for listeriosis are: pregnant women/fetus - perinatal and neonatal infections; persons immunocompromised by corticosteroids, anticancer drugs, graft suppression therapy, cancer patients - leukemic patients particularly; less frequently reported - diabetic, cirrhotic, asthmatic, and ulcerative colitis patients; the elderly; normal people - some reports suggest that normal, healthy people are at risk, although antacids or cimetidine may predispose. A

listeriosis outbreak in Switzerland involving cheese suggested that healthy uncompromised individuals could develop the disease, particularly if the foodstuff was heavily contaminated with the organism (CDC, 1994).

Food analysis

The methods for microbial analysis of food are complex and time consuming. The present FDA method, revised in September, 1990, requires 24 and 48 hours of enrichment, followed by a variety of other tests. Total time to identification is from 5 to 7 days, but the recent announcement of specific nonradiolabeled DNA probes should soon allow a simpler and faster confirmation of suspect isolates.

Recombinant DNA technology may even permit 2-3 day positive analysis in the future. Currently, FDA is collaborating in adapting its methodology to quantitate very low numbers of the organisms in foods (Seeliger and Jones, 1994).

Recent outbreaks

Recent outbreaks include the California episode in 1985, which was due to Mexican-style cheese and

led to numerous stillbirths. As a result of this episode, FDA has been monitoring domestic and imported cheeses and has taken numerous actions to remove these products from the market when *L. monocytogenes* is found.

There have been other clustered cases, such as in Philadelphia, PA. Specific food linkages were only made epidemiologically in this cluster. CDC has established an epidemiological link between consumption of raw hot dogs or undercooked chicken in approximately 20% of samples (CDC, 1995).

CHAPTER 3
LETHALITY OF CHEMICALS TO *Listeria monocytogenes*
Scott A: D-VALUE DETERMINATIONS IN TSB AND CATFISH
FILLETS UTILIZING 2% LACTIC ACID, 2% ACETIC ACID,
1% HYDROGEN PEROXIDE & 50ppm SODIUM HYPOCHLORITE

Introduction

D-values for *Listeria monocytogenes* strain Scott A were determined in tryptic soy broth (TSB) (Difco, 1995), inocula $\sim 1.0 \times 10^3$ and 1.0×10^6 and inoculated channel catfish (*Ictalurus punctatus*) fillets inoculated at $\sim 1.0 \times 10^3$. Chemicals utilized for killing *L. monocytogenes* were 2% lactic acid (LAc), 2% acetic acid (HAc), 1% hydrogen peroxide (HP) and 50ppm sodium hypochlorite (SH). Concentrated chemicals were added to broths so that the medium was instantly raised to the desired concentration. One milliliter aliquotes were taken every minute for ten minutes. Each aliquot was immediately pipetted into a 99ml 1% peptone dilution bottle, serially diluted and plated on tryptic soy agar (TSA).

Catfish fillet nuggets were incubated in a TSB *L. monocytogenes* broth, concentration $\sim 1.0 \times 10^3$, for 30 min. Inoculated nuggets were immersed in the appropriate chemical bath, and samples removed every

two minutes, stomached and serially diluted. Dilutions were plated onto TSA for general APC counts. *L. monocytogenes* survival was determined by plating dilutions onto media; media specifically formulated for selecting *Listeria spp* (McBride's Agar, 5% sheep blood and 0.02% cyclohexamide).

Since *Listeria*'s emergence as a human pathogen, surveys have found the species ubiquitous in nature (Gellin et al., 1987). One study indicated as much as 61% of fish and seafoods to exhibit *Listeria* presence (Weagnat et al., 1988). Shewan and Hobbs suggested that bacterial flora on fish reflected its environment. A 1.99 log CFU/g *Listeria* was found in the viscera of channel catfish (Leung et al., 1995) and be concluded that cross contamination to flesh was possible. Contamination of fish filets during processing is unavoidable. Raw or cooked catfish / shrimp supported *Listeria* growth significantly better than beef or chicken (Shinemann and Harrison 1994). Rawles et al. (1995a) found 10% of blue crabs in processing plants were infected with *Listeria spp*.

The spoilage and poisoning of foods by microbes is a problem that is worldwide. The susceptibility of aquaculture and marine species is of special concern because of the growing consumer demand for low fat/high protein commodities. Catfish is the most successfully aquacultured species in the United States and is gaining a growing consumer and restaurant market share (Dellenbarger et al., 1988; Israel et al., 1993). With an increasing emphasis on "natural/fresh" product, processors often utilize only mild, if any, further preservation methods.

Processed catfish could be dipped into chemical solutions to lower the overall bacterial number and reduce or eliminate *L. monocytogenes*. Immersion time needed to achieve significant bacterial inhibition has not yet been established. However, the use of organic acids as dips are desirable because of their common acceptance with the consumer, effectiveness and standing as GRAS substances.

The pH at which *Listeria* is inhibited is yet to be definitively determined. *Listeria* failed to grow in a dextrose broth, pH 5.6 (Seeliger and Finger,

1976) or in a silage medium, pH 5.5 (Irvin 1969). *L. monocytogenes* was shown to grow in clarified cabbage juice with a pH of 5.6 at 5°C. Viable cells were detected at day 35 with a pH of 4.8. Ahamad and Marth (1989) found acetic acid mediums inhibited *L. monocytogenes* to a much greater degree than lactic acid.

Fletcher et al. (1993) reported that a 1% hydrogen peroxide solution resulted in complete elimination of *Salmonella typhimurium* with a concentration of 1.0×10^7 in fifteen minutes. Chicken legs were artificially inoculated, incubated and then dipped into an unknown solution of concentration of hydrogen peroxide achieving a 2 log reduction in bacteria.

Sodium hypochlorite is widely used in dips in the poultry industry. While not extremely effective, unless used in high concentration, sodium hypochlorite is commonly used and was also investigated in this study.

The first objective of this work was to determine the D-values for *L. monocytogenes* in two

pure culture concentrations. A 1.0×10^3 CFU/ml, a relatively low level, and 1.0×10^6 CFU/ml, simulating spoilage conditions for a variety of foods. A second objective was to determine the efficacy of 2% acetic acid, 2% lactic acid, 1% hydrogen peroxide and 50 ppm sodium hypochlorite in eliminating *L. monocytogenes* from an inoculated catfish as compared to pure culture.

Materials and methods

The following information details the materials and procedures utilized in the experiment previously detailed.

Broth & culture preparation

Listeria monocytogenes strain Scott A was used in the study. Stock cultures were maintained through monthly transfers on tryptic soy agar (TSA - Difco, 1995) and stored at 23°C. Before each survival curve was determined, an inoculum from a stock culture was transferred into a tube of tryptic soy broth (TSB - Difco, 1995) and incubated for 24hr at 30°C for three consecutive days. Daily APC indicated 1.0×10^8 CFU/ml. Three quarters (0.75ml) of the 24 hour broth

was aseptically pipetted into 750ml TSB yielded an inocula of $\sim 1.0 \times 10^6$. One loop aseptically transferred into 750ml TSB yielded an inocula of $\sim 1.0 \times 10^3$. Broths were stirred for 5 minutes before the chemicals were added.

Catfish preparation & treatment

Fresh channel catfish (*Ictalurus punctatus*) were purchased from a retail source immediately following dressing. The fillets were taken to Louisiana State University on ice and used within the hour. Fillets were cut into 10g cubes and placed in TSB contaminated with *L. monocytogenes* at 1.0×10^3 and allowed to incubate for 30 minutes. Nuggets were removed from TSB and immediately immersed into the appropriate 750ml chemical bath at 23°C.

Treatment procedures & microbiological analysis

Broths of the appropriate *L. monocytogenes* concentration were plated to represent time 0. A volume of concentrated chemical was added to the TSB culture and a one milliliter aliquot was removed and pipetted into a 99ml of a 1% peptone dilution blank. Serial dilutions were made in duplicate and TSA pour

plates were made and incubated for 24 hours at 30°C. The entire procedure was performed in triplicate for each chemical and microbial concentration.

Treated fillet nuggets were immersed into a 750ml chemical bath. One nugget was plated immediately before treatment. Once treatment began, samples were removed every two minutes. At the appropriate time, samples were removed, weighed and 9% sterile peptone water was added to produce a 1:10 dilution (wt/vol). A Tekmar 400 Series Stomacher was used to homogenize the nugget for 1 minute. Serial dilutions were made of the homogenate on TSA in duplicate. To determine the *L. monocytogenes* survivors, aliquots were plated onto McBride's Agar supplemented with 0.2% cyclohexamide. The entire procedure was performed in triplicate for each chemical tested. All plates were incubated at 30°C for 24 hours.

Chemical preparation

None of the chemicals used as treatments originated in the TSB. As a result a simple dilution scheme was utilized to dilute the concentrated

chemical into the TSB for the desired concentration. A similar procedure utilized water to dilute the concentrated chemicals to the appropriate percentages for the fillet dip bath at 23°C.

Sodium hypochlorite was diluted to 50ppm but was not acidulated to induce hypochlorous acid. This procedure was adopted to simulate processing and cleaning procedures commonly found in seafood processing plants.

Results and discussion

The results and discussion are broken into groups. The first group is the establishment of a linear relationship to broth and flesh or dynamic systems.

D-Value determinations

Listeria monocytogenes survival curves for both broth concentrations and inoculated nuggets are presented in Figures 7, 8, 9 and 10 for LAc, HAc, SH and HP respectively. Figure 11 shows the general APC decline over time. Summary regression statistics and D-values are listed in Table 4.

Table 4. D-values for *L. monocytogenes* at different concentrations and chemical additives

Chem.	Media	R ²	Slope	Y-intercept log CFU/g	D-Value ^o (min)
LAc broth pH 2.69					
LAc	TSB ee3	0.92	-0.092	2.96	11.38 ^a
LAc	TSB ee6	0.92	-0.049	6.11	24.25 ^a
LAc	FISH	0.82	-0.180	2.68	5.54 ^a
HAc broth pH 3.54					
HAc	TSB ee3	0.94	-0.430	3.63	2.43 ^a
HAc	TSB ee6	0.96	-0.089	6.021	11.29 ^a
HAc	FISH	0.96	-0.116	3.11	8.57 ^a
SH broth pH 7.08					
SH	TSB ee3	0.24	xx	xx	xx
SH	TSB ee6	0.30	xx	xx	xx
SH	FISH	0.57	xx	xx	xx
HP broth pH 7.02					
HP	TSB ee3	**	**	**	** ^b
HP	TSB ee6	0.88	-0.164	6.27	6.12 ^a
HP	FISH	0.98	-0.195	3.21	5.21 ^a

^{a, b} different letters indicate significant differences
Tukey Groupings (P<0.05)

xx R² too poor to model

** no curve established, bacteria destroyed

◊ D-value calculated as -1/m

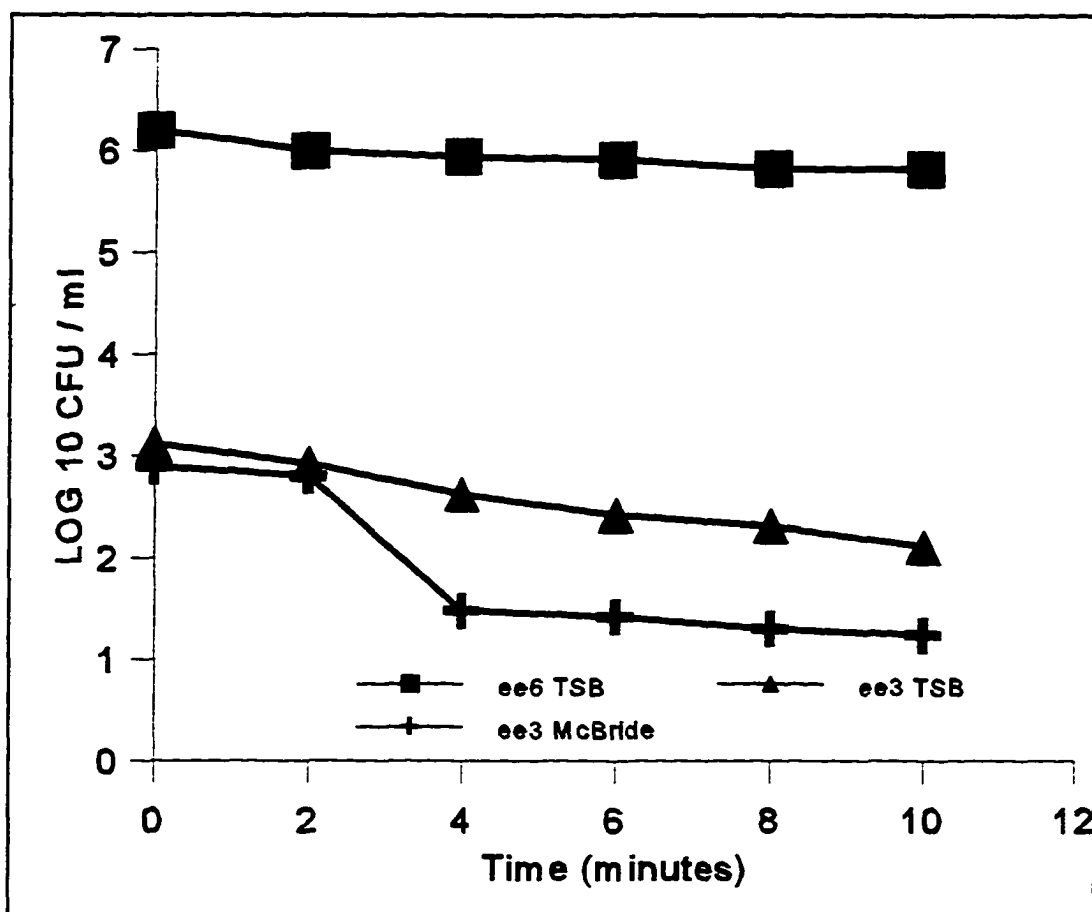


Figure 7. Chemical death survival curves for *L. monocytogenes*, TSB and fillets acidulated to 2% lactic acid.

Tukey groupings ($P < 0.05$) indicate no significant D-value differences with 2% lactic or acetic acid acidulation. The bacterial log reductions are statistically the same for a planktonic culture simulating spoilage counts, possible infections numbers and inoculated fillets. Flesh proteins did not yield protection to inoculated bacteria.

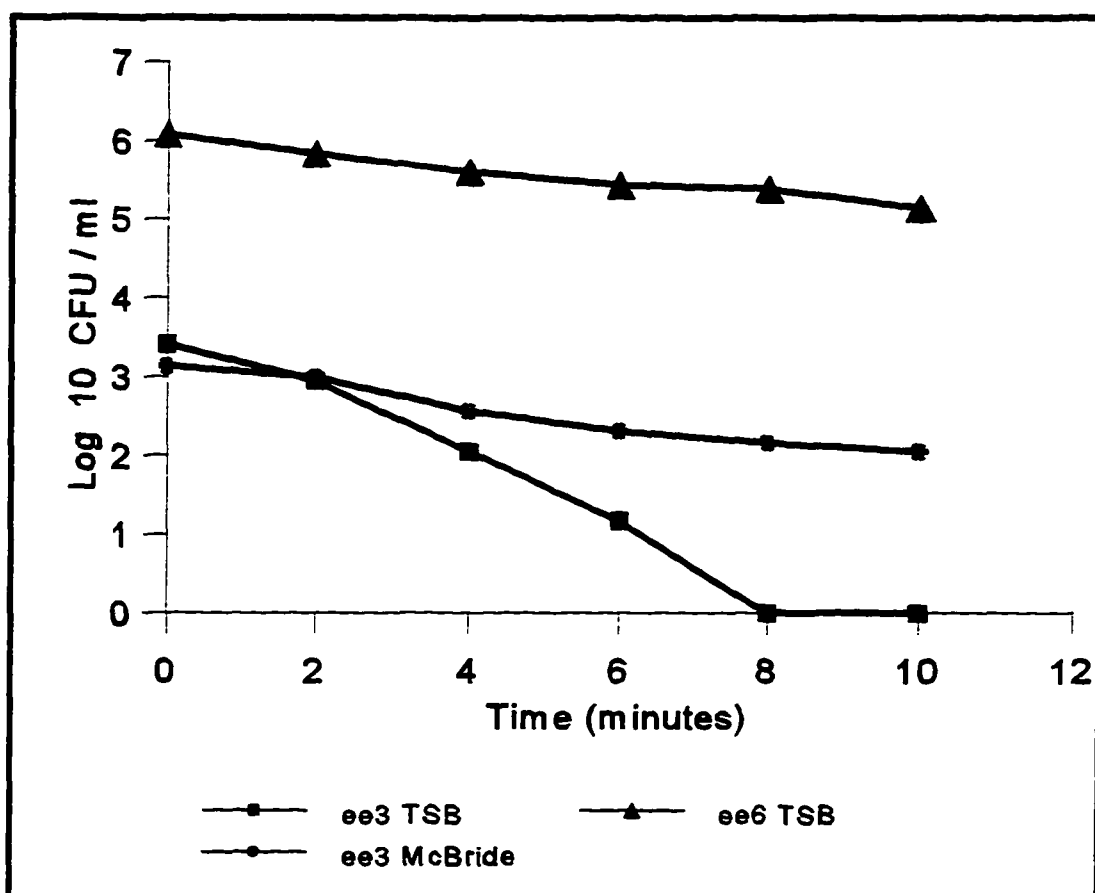


Figure 8. Chemical death survival curves for *L. monocytogenes*, TSB and fillets acidulated to 2% acetic acid.

Tukey groupings ($P < 0.05$) indicate no significant D-value differences with 2% lactic acid acidulation. The bacterial log reductions are statistically the same for a planktonic culture simulating spoilage counts, possible infections numbers and inoculated fillets. Flesh proteins did not yield protection to inoculated bacteria. However at 8 minutes, elimination of

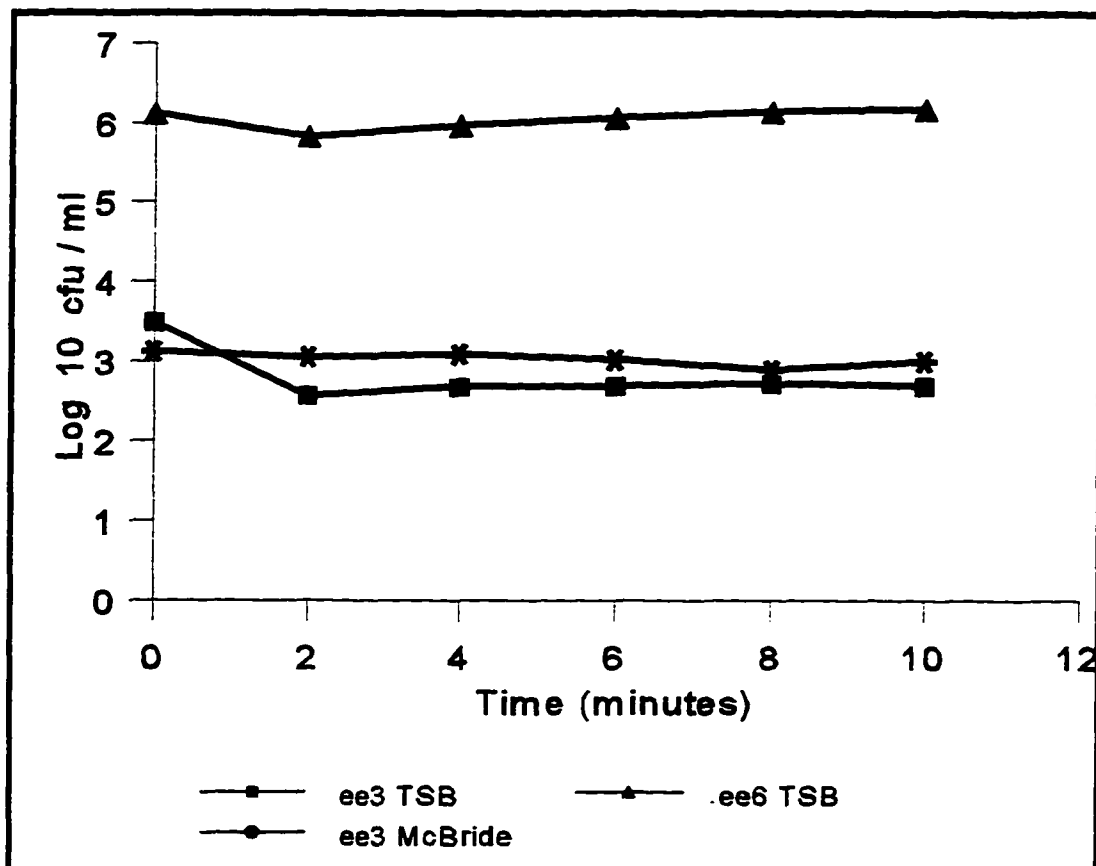


Figure 9. Chemical death survival curves for *L. monocytogenes*, TSB and fillets treated with 50ppm sodium hypochlorite.

L. monocytogenes at $\sim 1.0 \times 10^3$ was achieved. Least Squares regression correlations were so poor, mathematical models were not possible. However, no detrimental effects were seen. Planktonic cells at the spoilage level actually possess a positive slope.

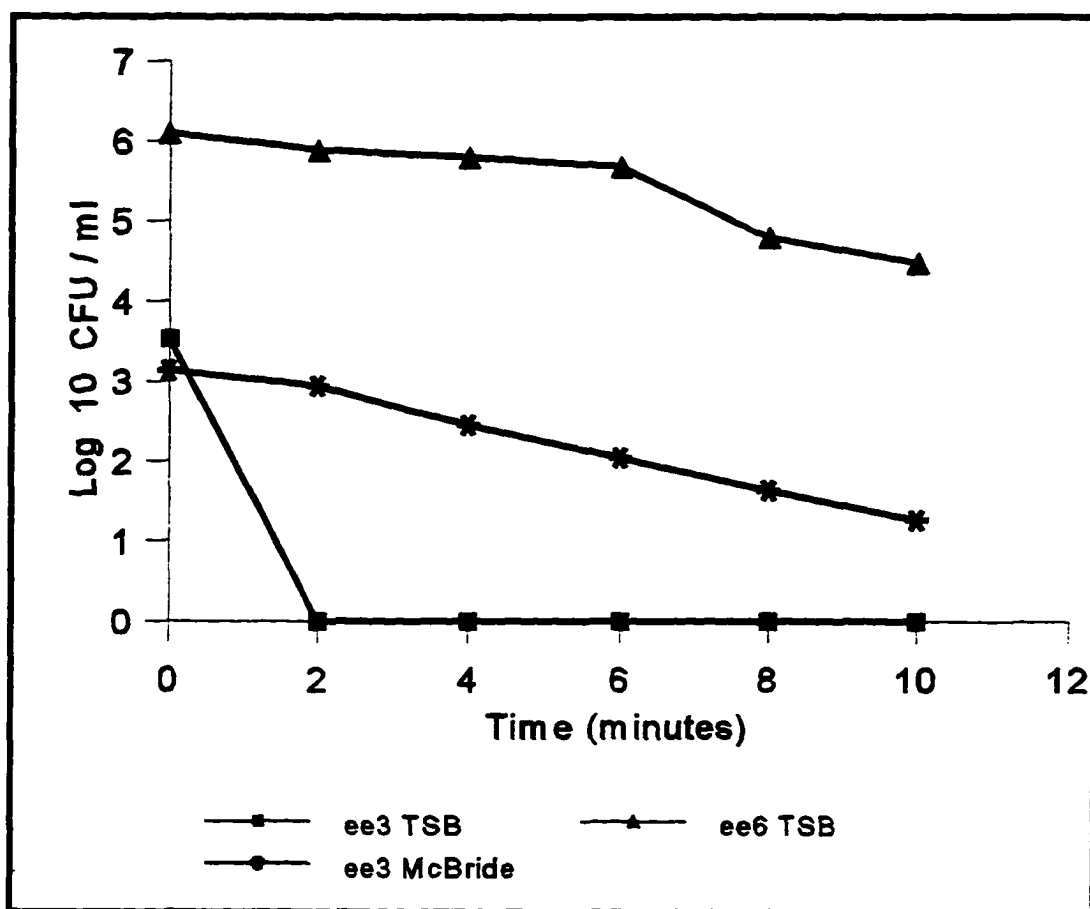


Figure 10. Chemical death survival curves for *L. monocytogenes*, TSB and fillets treated with 1% hydrogen peroxide.

Hydrogen peroxide affected a near instantaneous elimination of *L. monocytogenes*. Tukey groupings ($P < 0.05$) indicate no significant D-value differences with 1% hydrogen peroxide. The bacterial log reductions are statistically the same for a planktonic culture simulating spoilage counts, possible infections numbers and inoculated fillets.

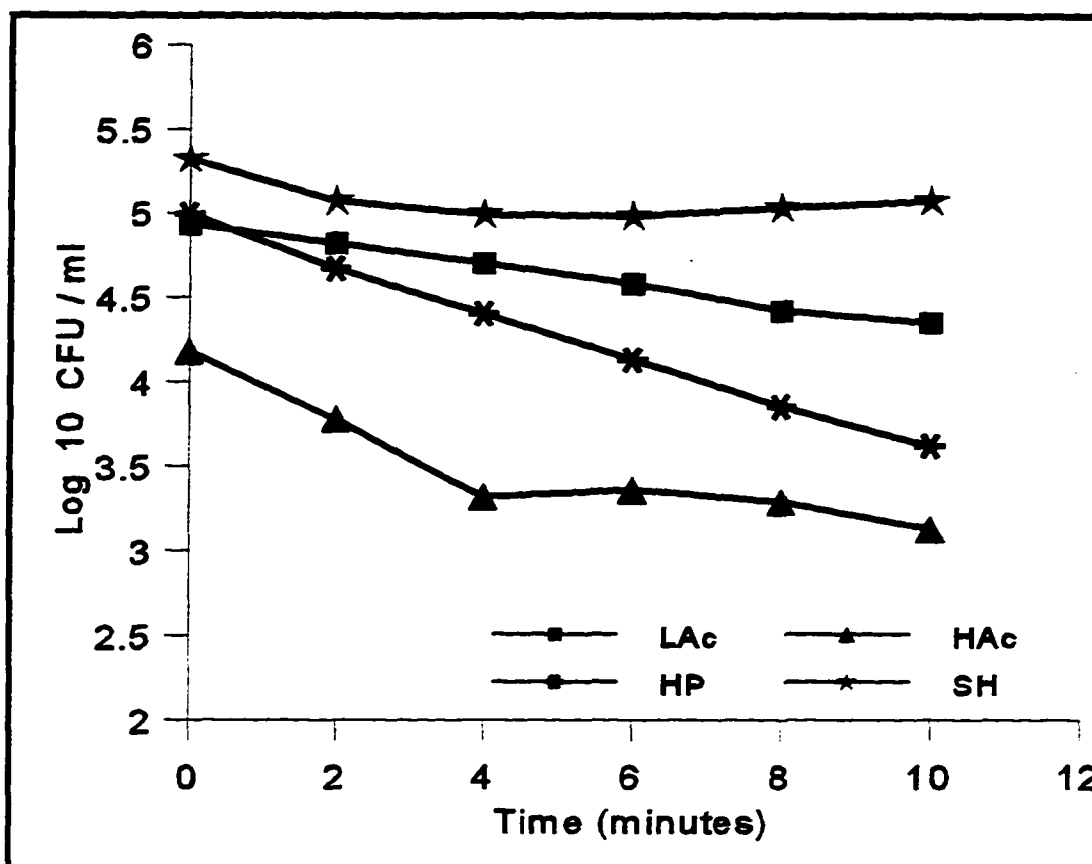


Figure 11. Chemical death survival curves for mixed flora of fillets for chemical treatments.

While some survival curves seen to suggest a greater bacteriocidal effect, Least Squares regressions indicate no rate or D-value difference exists between treatment or levels with two exceptions. Hydrogen peroxide's exhibited near instant reduction of *L. monocytogenes* where sodium hypochlorite had no significant effect on *Listeria*. Only in hydrogen peroxide treatments did catfish flesh significantly shield *Listeria*.

CHAPTER 4
EXTENDING SHELF LIFE OF CATFISH FILLETS ON ICE, USING
HOT HYDROGEN PEROXIDE, ACETIC OR LACTIC ACID ALONE
AND IN COMBINATION & THEIR EFFECTS ON
Listeria monocytogenes

Introduction

The effects of immersing fresh catfish fillets in 1% hydrogen peroxide (HP), 2% acetic acid (HAc), 2% lactic acid (LAc), 2% acetic / 1% peroxide (HAHP), 2% lactic / 1% peroxide (LAHP) dips heated to 65°C for 30 seconds followed by 50ppm sodium hypochlorite ice drench was investigated. Changes in total aerobic plate counts (APC), *Listeria monocytogenes* Scott A counts (LMC) and pH values of catfish fillets, during storage on ice at 4°C for 12 days, were determined. Initial dipping of fillets in HP reduced initial microbial loads by 1.88 logs, dips in HAc and LAc reduced initial microbial loads, respectively, by 1.61 / 0.44 logs and HAc&HP or LAc&HP dips lowered microbial counts by 1.79 / 1.61 logs compared to controls. All treatments had significantly lower APC's after 12 days compared to controls. HAc was found to have been the most

effective by decreasing APC counts to 5.06 compared to controls (log counts 8.4×10^8) at day 12.

Listeria monocytogenes counts were determined by inoculation of fillets with *Listeria monocytogenes* Scott A, $\sim 1.0 \times 10^3$, for a period of 30 minutes and noninoculated samples were treated in a similar fashion. *Listeria monocytogenes* was recovered and enumerated on McBride's Agar. All treatments suppressed bacterial expression on McBride's media at day 0 and 3. HAC was found to be the most effective treatment to inhibit *L. monocytogenes*, with a log count reduction of 3.34 after 12 days storage. The pH values for stored and treated fillets, utilizing organic acids, were found to have significantly lower pH's than the control or HP alone through out the storage study.

The spoilage and induced pathogenicity of foods by microbes is a problem that is of world wide nature. The susceptibility of aquaculture and marine species is of special concern because of the growing consumer demand for low fat/high protein commodities. Catfish is the most successfully aquacultured species

in the United States and is gaining a growing consumer and restaurant market share (Dellenbarger et al., 1988 and Israel, 1993). With an increasing emphasis on "natural/fresh" products, processors often utilize as mild preservation methods as possible.

The use of organic acids are desirable because of their common acceptance with the consumer, effectiveness and standing as GRAS substances. The pH at which *Listeria* is inhibited is unclear. *Listeria* failed to grow in a dextrose broth with a pH of 5.6 (Seeliger and Finger, 1976) or in a silage medium, pH 5.5 (Irvin, 1969). *L. monocytogenes* was shown to grow in clarified cabbage juice with a pH of 5.6 at 5°C. Viable cells were recovered from the cabbage juice after day 35 at a pH of 4.8 (Conner et al., 1986). Ahamad and Marth (1989) found acetic acid mediums inhibited *L. monocytogenes* to a much greater degree than lactic acid.

Fletcher et al., 1993, reported that a 1% hydrogen peroxide solution in conjunction with sodium hydrogen carbonate resulted in complete elimination

of *Salmonella typhimurium* with a concentration of 1.0×10^7 CFU in fifteen minutes. Chicken legs were artificially inoculated and placed in an undisclosed solution concentration of hydrogen peroxide and achieved a bacterial reduction of 2 logs.

At 55°C, both catalase and superoxide dimutase activity was arrested for *Escherichia coli* 0157:H57. Overall microbial counts were reduced.

Prior to the 1980's, *Listeria spp.* were pathogens that primarily affected livestock causing spontaneous abortions and mastitis (Amstutz, 1980). Since the 1980's, listeriosis in humans has caused several food poisoning outbreaks and deaths. *Listeria spp.* usually affects the young, old and immunocompromised with a suspected infectious dose of $\sim 1.0 \times 10^6$ (Bruce and Broome, 1989; Schlech et al., 1983).

Since *Listeria's* emergence as a human pathogen, surveys have found the species ubiquitous in nature (Gellin et al., 1987). Weagant et al. (1988) surveyed frozen seafood retailers and found as high as 61% of fish and seafood to contain *Listeria spp.*

Wyatt et al., (1979) suggested that bacterial flora on fish reflected its environment. A 1.99 log CFU/g *Listeria* were found in the viscera of channel catfish (Leung et al., 1995) and concluded that a cross contamination to flesh was possible. Contamination of fish filets during processing is unavoidable. Raw or cooked catfish / shrimp supported *Listeria* growth significantly better than beef or chicken (Shinemann and Harrison, 1994). *Listeria* (1.0×10^4 CFU) was applied to the finger tips of workers and was not removed with washing using soap or a chlorhexidine hand cleanser. *Listeria* numbers persisted on the finger tips for well over an hour (Snelling, 1991). Food workers were found to have a significantly greater chance of carrying *Listeria* spp. than clerical workers ($P < 0.015$) (Kerr, 1993).

Listeria monocytogenes has established itself as one of the few psychrotrophic pathogens (Fleming et al., 1985). The possibility of natural as well as food handler contamination of food coupled with the ability to proliferate at refrigeration temperatures could represent a significant health hazard.

Materials and methods

The following information details the materials and procedures utilized in the experiment previously detailed.

Catfish preparation and treatment

Fresh channel catfish (*Ictalurus punctatus*) were purchased from a retail source immediately following dressing. The fillets were taken to Louisiana State University Food Science Laboratory on ice and further processed within the hour. Two liter hot water baths (65°C) were prepared and the food preservatives were added separately and in combination. Fillets were dipped for 30 sec., removed and immediately placed in a sodium hypochlorite ice water slush (50ppm nonacidulated to simulate common processing practices, solution pH 7.2). The fillets were placed in 8" x 12" polyethylene bags, sealed and stored on ice in a 4°C walk-in cooler. Untreated fillets were sealed in bags and immediately stored on ice. Triplicate experimental trials, were performed in duplicate with the following protocol: experimental dips 2% HAC (solution pH 3.54), 2% Lac (solution pH

2.96), 1% HP (solution pH 7.02), 2% HAc & 1% HP (solution pH 3.54), 2% LAc & 1% HP (solution pH 2.96).

Additional fillets were inoculated with approximately 1.0×10^3 CFU/cc in a tryptic soy broth of *Listeria monocytogenes* for thirty minutes. Inoculated fillets were then processed in a similar manner to noninoculated fillets. Microbiological counts and pH readings of treated fillets stored on ice at 4°C were taken every three days over a 12 day storage period.

Microbiological analysis

Each fillet was weighed and 9% sterile peptone water was added to make a 1:10 dilution (wt/vol). A Tekmar 400 Series Stomacher was used to homogenize the fillet for 1 minute.

Serial dilutions were performed in duplicate on Tryptic Soy Agar (TSA) for the noninoculated fillets. Fillet homogenates, *Listeria* inoculated, were plated onto McBride's Agar supplemented with 5% sheep's blood and 0.2% cycloheximide (Difco, 1995). All plates were incubated at 30°C for 24 hours.

Measurement of pH

Fillet pH was measured with a standardized surface pH electrode and pH meter (Orion A983) by placing the surface electrode directly on to the fillet surface. The pH values are reported as the average of triplicate readings for each fillet.

Preparation of cultures

Listeria monocytogenes Scott A was used in the study and obtained from the Louisiana State University Food Science stock cultures. Stock cultures were maintained through monthly transfers on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) and stored at 23°C. To prepare for each experiment, inoculum from a stock culture was transferred to a tube of TSB which was incubated for 24 hr at 30°C. This was followed by two more similar transfers in TSB on two consecutive days. *L. monocytogenes* broth was appropriately diluted into 500ml of TSB and inoculated test samples so that the initial CFU/ml was either 1×10^3 or 1×10^6 .

Results and Discussion

Mean pH values were measured on catfish fillets dipped at 65°C for 30 seconds in 2% LAc, 2% HAc,

1%HP, 2%LAc and 1%HP, 2%HAc and 1%HP during storage on ice at 4°C. All treatments resulted in lower pH values when compared to controls (control¹ no heat treatment and control² 65°C water treatment only) except for hydrogen peroxide on day 3. Table 5 shows the mean pH value for the various treatments and storage periods.

Table 5. Comparison of mean pH values for various treatments for catfish fillets stored on ice at 4°C over 12 days.

	Storage Time (days)				
Treatment	0	3	6	9	12
Contro ¹	6.24 ^a	6.49 ^a	6.97 ^a	7.38 ^a	7.91 ^a
Control ²	6.00 ^a	6.40 ^a	6.82 ^a	7.42 ^a	7.89 ^a
1% HP	6.35 ^b	6.51 ^a	6.64 ^b	6.79 ^b	7.04 ^b
LAc-HP	4.01 ^c	4.29 ^b	4.50 ^c	5.41 ^c	6.00 ^c
2% LAc	3.96 ^c	4.12 ^c	4.45 ^c	5.34 ^c	5.85 ^{cd}
HAc-HP	3.37 ^e	3.85 ^d	4.41 ^c	4.84 ^d	5.65 ^d
2% HAc	3.60 ^d	4.23 ^{bc}	4.31 ^d	4.68 ^e	5.37 ^e

a,b,c,d,e Means in the same column with different superscripts are significantly different ($P < 0.05$).

Hydrogen peroxide and acetic acid did significantly lower the pH compared to controls, but as expected, was significantly higher compared to the acid treatments. All pH's significantly increased at the end of the storage study compared to initial pH.

Except for day 3, there was no significant pH difference in samples treated with lactic acid and the lactic/peroxide combination.

In the first half of the storage period, the samples treated with HAc and in combination with HP maintained a lower sample pH than those treated with HAc alone. In the latter half, the HAc treated samples maintained a lower sample pH than those treated with HAc/HP. Figure 12 shows acetic and lactic acids treated samples over time. Figure 13 shows fillet's of the GRAS acid treatments in combination with HP and HP's effects on the sample pH alone.

All treatments demonstrated a significant reduction of mean log aerobic plate counts (APC) over the storage period (12 days on ice at 4°C) compared to controls. Table 6 shows the suppression in microbial growth over time by day and treatment for the 12 day storage period.

Except for day 0, there was no significant microbial reduction between the LAc and LAc/HP treated samples. The addition of HP to the LAc bath did not exhibit an additive effect.

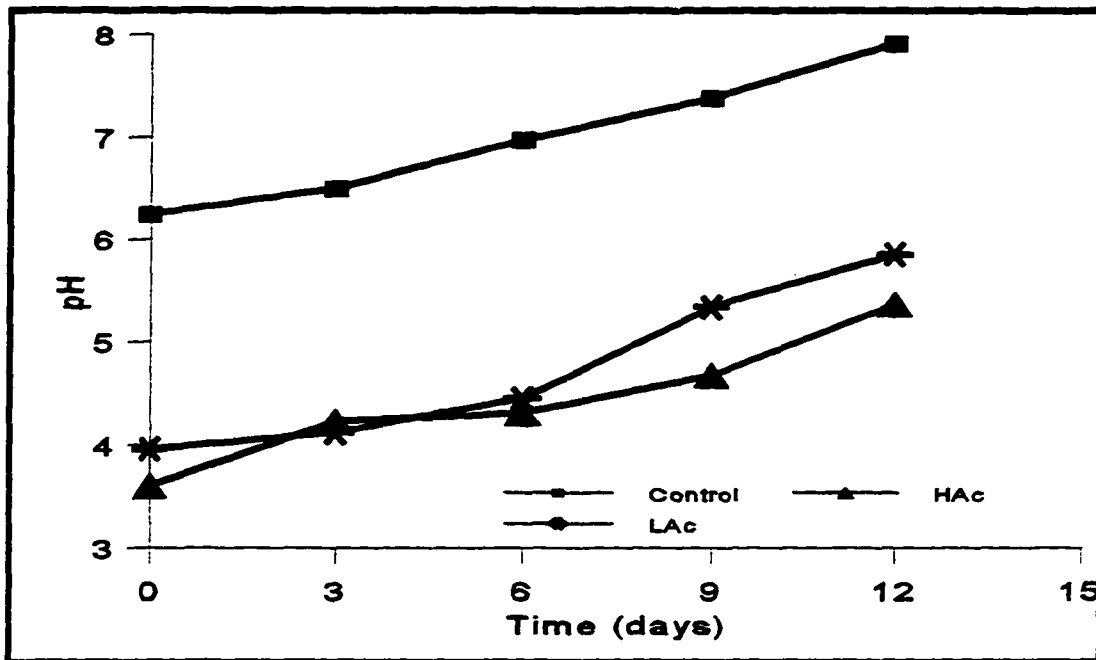


Figure 12. pH of catfish fillets stored 12 days on ice after treatment (HAc and LAc at 65°C, 30 sec.)

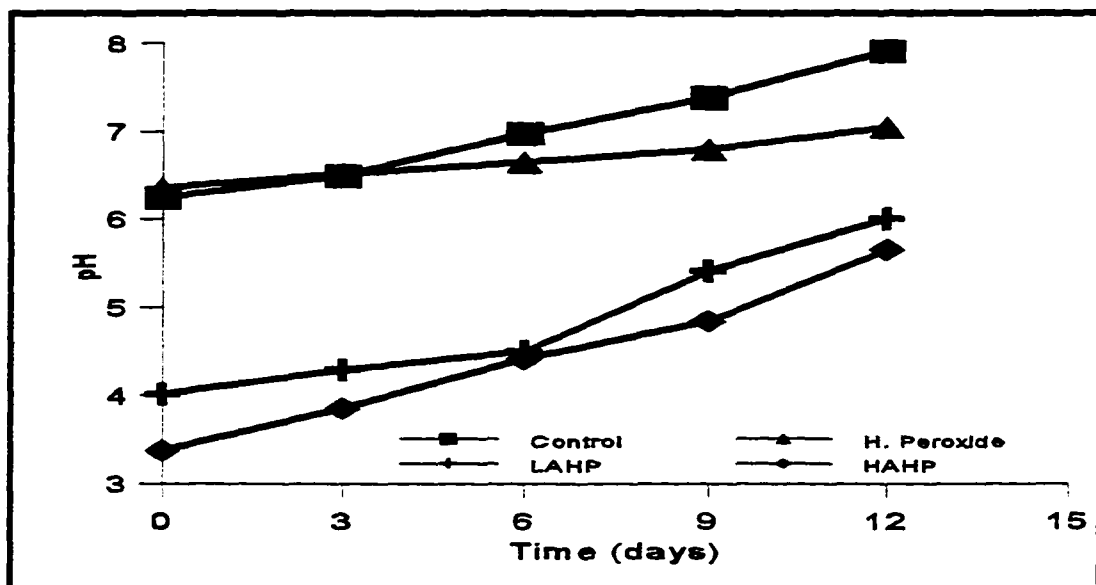


Figure 13. pH of catfish fillets stored over time on ice after treatment (HP combined with HAc and LAc 65°C, 30 sec.)

HAc was more effective than LAc in reducing bacterial growth. Fillet treatment with HAc exhibited consistently lower APC counts on each sampling days compared to fillets treated with LAc. Figure 14 illustrates the APC for fillets treated with HAc and LAc compared to control fillets over time. The addition of HP to the HAc bath did exhibit a significant antimicrobial effect than HAc alone on all sampling days but day 12. On day 12, there was no significant difference between the two treatments.

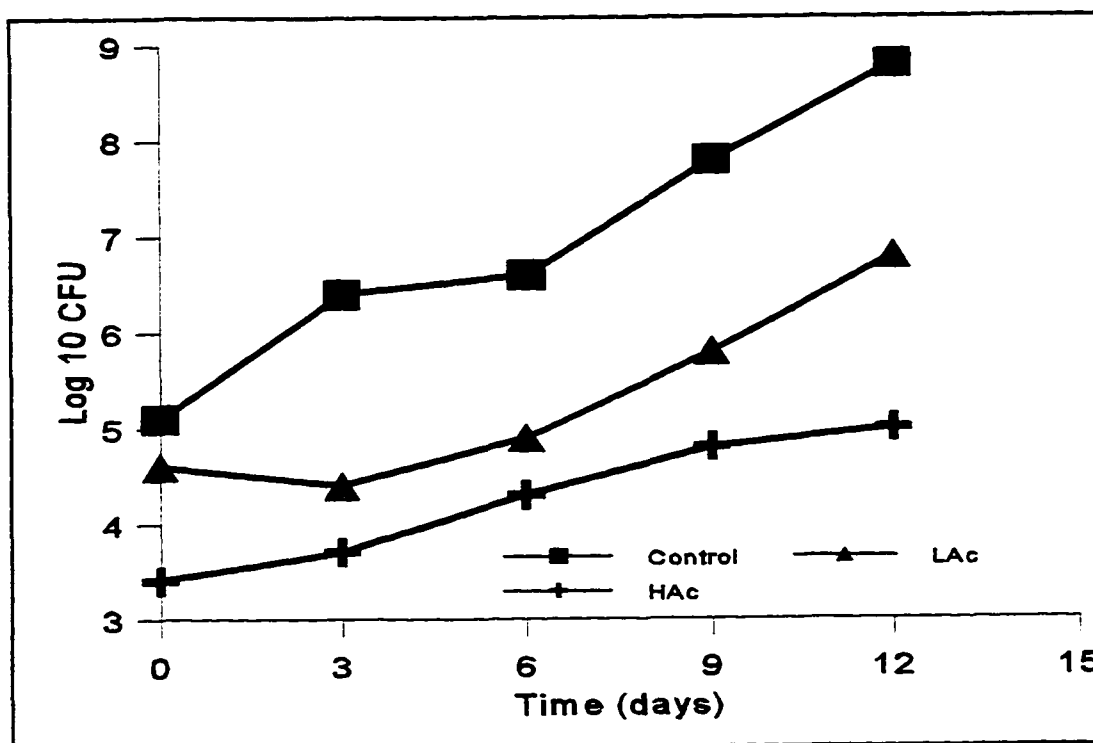


Figure 14. Mean APC counts of catfish fillets stored over time on ice after treatment (HAc and LAc 65°C, 30 sec.).

Figure 15 illustrates the APC for fillets treated

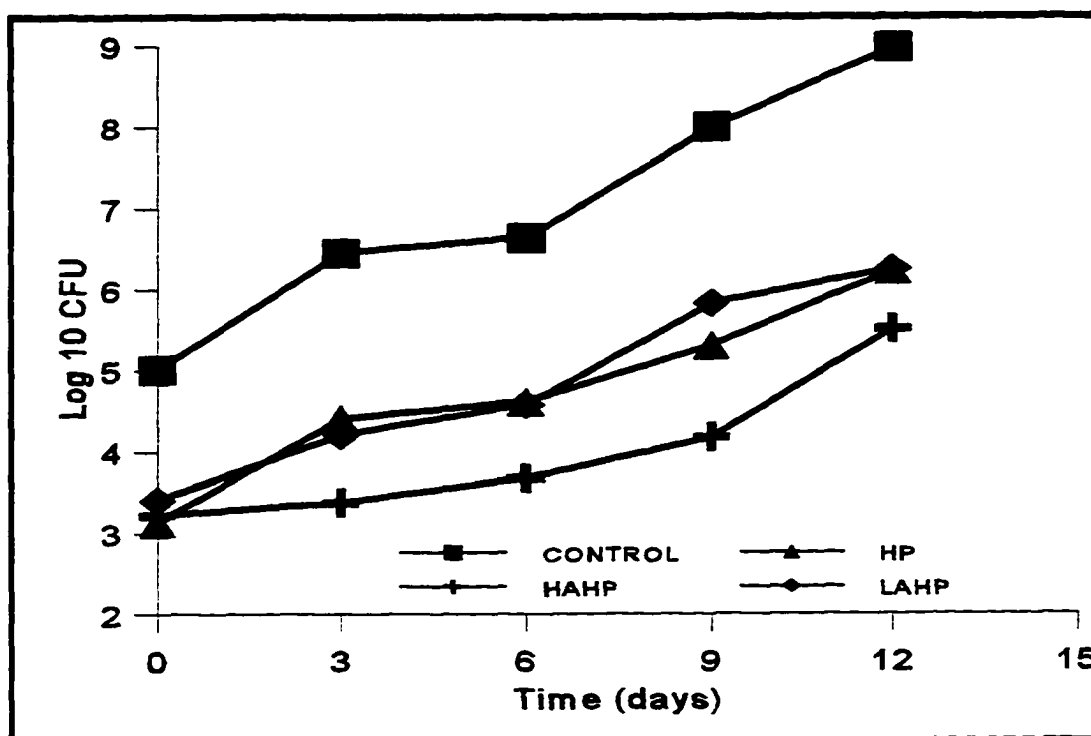


Figure 15. Mean APC counts during storage utilizing hydrogen peroxide and organic acids in combination (65°C, 30 sec.).

Table 6. Comparison of shelf life extension over 12 days with different chemical treatments, mean log APC.

	Storage Time (days)				
	0	3	6	9	12
Control	5.00 ^a	6.44 ^a	6.64 ^a	7.99 ^a	8.98 ^a
HP	3.13 ^c	4.39 ^b	4.61 ^{bc}	5.31 ^c	6.22 ^b
LAc	4.56 ^b	4.42 ^b	4.82 ^b	5.76 ^b	6.69 ^b
LAcHP	3.40 ^c	4.20 ^b	4.57 ^{bc}	5.82 ^b	6.25 ^b
HAHP	3.22 ^c	3.36 ^c	3.67 ^d	4.16 ^e	5.51 ^c
HAc	3.39 ^c	3.54 ^c	4.38 ^c	4.66 ^d	5.05 ^c

a, b, c, d, e Different letters in the same column indicate significant differences Bonferroni T test ($P < 0.01$).

with HP and in combination with LAc and HAc compared to controls over time.

Hydrogen peroxide was the most effective in lowering bacterial numbers on day 0. Over time, peroxide in conjugation with lactic acid exerted no significant additional suppression, but did with HAc.

All treatments exerted a significant reduction of *L. monocytogenes* inoculated fillets compared to the controls. Table 7 shows the suppression of *L. monocytogenes* by day and treatment over the twelve day storage period.

After treatment, no *L. monocytogenes* were detected on day 0 and 3. No *L. monocytogenes* was detected on day 6 with HAc treated samples.

Table 7. Comparison of *Listeria monocytogenes* suppression on inoculated fillets stored over time on ice by chemical treatment by day.

Treatment	Storage Time (days)				
	0	3	6	9	12
Control	3.08 ^a	3.23 ^a	3.72 ^a	4.73 ^a	6.54 ^a
1% HP	0.00 ^b	0.00 ^b	1.70 ^b	2.68 ^{bc}	4.51 ^d
LAc-HP	0.00 ^b	0.00 ^b	1.42 ^b	3.38 ^b	5.23 ^c
2% LAc	0.00 ^b	0.00 ^b	1.00 ^b	2.68 ^{bc}	4.51 ^d
HAc-HP	0.00 ^b	0.00 ^b	0.82 ^b	2.13 ^c	3.27 ^e
2% HAc	0.00 ^b	0.00 ^b	0.00 ^c	2.10 ^c	2.91 ^e

a, b, c, d, e Different letters in the same column indicate

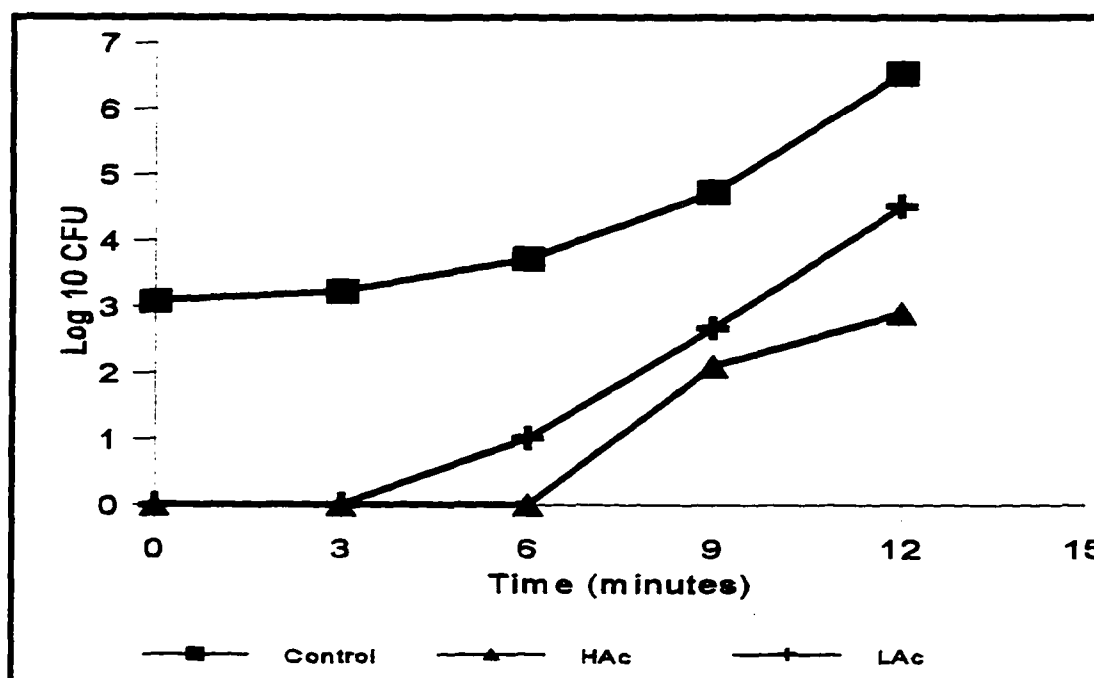


Figure 16. Mean McBride counts for *Listeria monocytogenes* inoculated fillets during storage on ice after treatment (LAc and HAC 65°C, 30 sec.).

HAC significantly lowered microbial counts on day 6 and 12 as compared to LAc. Figure 16 illustrates *L. monocytogenes* growth on fillets treated with lactic and acetic acid. Except for day 12, there was no significant difference in McBride counts for fillets treated with LAc and LAc/HP in combination. No significant difference was found for other treatments compared to HP except for day 12.

Except for day 6, there was no significant difference in McBride counts for fillets treated with HAC and HAC/HP combinations. Both treatments were

significantly better than HP except for day 9.

Figure 17 illustrates *L. monocytogenes* growth on fillets treated with peroxide and peroxide, organic

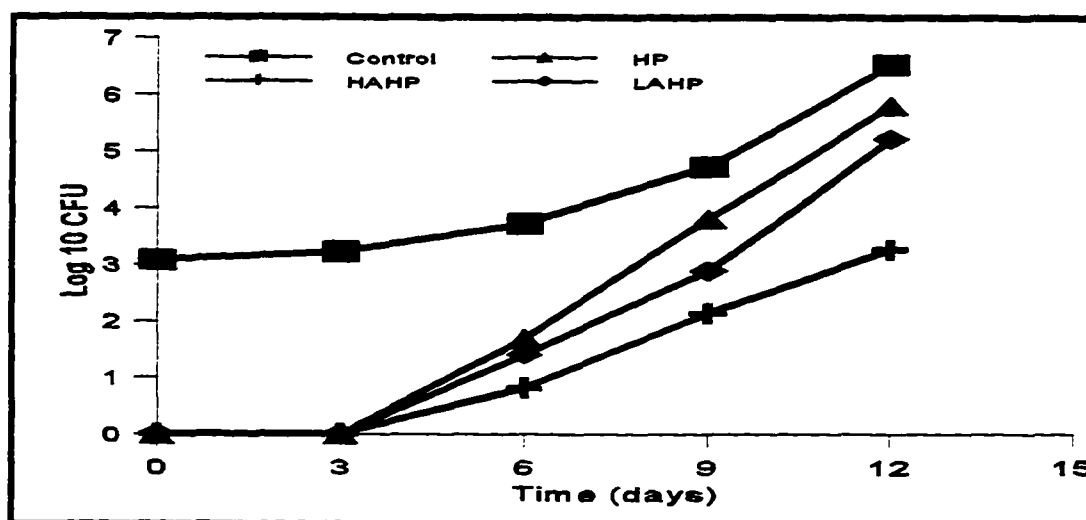


Figure 17. Mean McBride counts for *L. monocytogenes* inoculated fillets stored on ice after treatment utilizing hydrogen peroxide & organic acids in combination (HAc and LAc combined with HP 65°C, 30sec.).

acid combinations.

Organic acid sprays have been found to be effective in reducing bacterial populations. In this study, shelf life of catfish fillets was extended by use of lactic acid, acetic acid, hydrogen peroxide and acid/peroxide combinations. Surve et al. (1991) used a combination of 1.5% acetic and 1.5% lactic acid to extend the shelf life of buffalo meat. Osthold et al. (1984) found similar reductions with organic

acids sprays on beef and lamb carcasses. Treatments of fish and shellfish do not seem to have been studied.

While environmental acidulation can inhibit microbial growth, organic acids have been shown to be more detrimental than inorganic acids to growth. Organic acids work antimicrobially in three ways. First, organic acids can dissociate to a degree creating a high proton concentration. Second, very short chained acids, such as acetic, can be inserted into the bacterial membrane disrupting cohesion. In both cases, disruption of the cell's membrane, proton diffusion and lowering of the internal pH can cause death (Booth, 1985). Organic acids may act in a third way. Medium chain acids, such as lactic, can be transported into the cell where they can dissociate, lower the internal pH and cause cell death. Ahamad and Marth (1989) found *Listeria monocytogenes* Scott A survived at an external pH of 3.5, exposed to acetic and lactic acid. The intercellular fluid was lowered by 1 pH unit. Overall acidulation reached 5.0 and over a 24-hour period continued to decline. So, hydronium

concentration alone is not responsible for bacterial death.

Listeria monocytogenes has been eliminated by high temperature short time pasteurization of milk (73°C, 15 sec.). Pasteurization is dependant on time, temperature and microbial load. However, not all pasteurization trials eliminated *L. Monocytogenes* at 60°C, 15 sec and 3.0×10^6 CFU/ml. At these elevated temperatures, denaturation begins as well as the inactivation of enzymatic systems such as peroxide (Farber, 1991).

Hydrogen peroxide reduced the APC of chiller water from broiler plants by two log cycles at 0.06% (Lillard et al., 1987). All peroxides have the ability to oxidize any cell part and are extremely lethal.

In conclusion, organic acids and hydrogen peroxide are effective in reducing innate flora on fresh processed fish. The result is an extension of the storage period on ice. All treatments were effective against *Listeria monocytogenes*.

Initially, organic acids did not prove as effective as peroxides or peroxide/acid combinations.

Over time, acetic acid suppressed APC and McBride media counts most effectively. The additive effect of peroxide was not realized with lactic acid, but only initially so with acetic acid.

Until hydrogen peroxide is accepted in seafood and other product processing, as it is in Europe, the use of acetic acid alone is recommended. Further testing with other short chain acids and acid combinations with and without heat could bear further investigation.

CHAPTER 5
SENSORY ACCEPTABILITY
OF 65°C ACETIC ACID DIPPED CATFISH FILLETS

Introduction

A triangle difference test was used to determine if chemically treated channel catfish (*Ictalurus punctatus*) fillets were significantly different ($P < 0.05$) and acceptable to the consumer. Catfish fillets were immersed in a 65°C acetic acid (HAc) solution for 30 seconds, then cooled in a 50ppm sodium hypochlorite ice slush. Visual and olfactory detection of treated raw samples were measured. Samples similarly treated were baked at 204°C for 10 minutes (internal temperature 155°C). Triangle tests were employed to detect olfactory and gustatory differences in cooked samples. Overall product acceptability for raw and cook samples were also recorded.

Use and evaluation of sensory data in product processing is as important as the extension of shelf life (Civille, 1978). Discrimination tests are tests used to distinguish between samples or products. Discrimination tests that are most frequently utilized by U.S. food companies are the paired

comparison and triangle difference tests (Brandt, 1977). Dawson and Dochterman (1951) found no significant difference between paired comparison and triangle testing in confection comparison tests.

Materials and methods

The following information details the materials and procedures utilized in the experiment previously detailed.

Fillet preparation and treatment

Channel catfish (*Ictalurus punctatus*) were purchased and were dressed at a local retail seafood outlet. The fillets were transported to the Louisiana State University Food Science Processing Laboratory on ice and processed within two hours. Fillets were immersed for 30 seconds in a 65°C acetic acid solution. The fillets were then removed and immersed into a 50ppm sodium hypochlorite ice slush. Raw fillets were then placed with untreated samples in random order for visual and olfactory triangle difference testing.

Both treated and untreated fillets were cooked separately in a 204°C oven for 10 minutes (internal temperature 155°C). The fillets were cut into

approximately 1 cubic centimeter samples. The samples were then placed into cups in a random order for olfactory and gustatory triangle difference testing.

Panelist selection

Ten panelist that enjoy baked fish and consumed such at least biweekly were selected from the LSU faculty, students and staff. All panelists participated in an informal training session to introduce them to the procedures of performing a triangle test. Panelists were not told the species of fish or treatments that would be involved.

Triangle test design

Each panelist evaluated 4 cooked and 4 raw triangle test sets per session. Three sessions or replications were undertaken. Sample cups and fillets were randomly assigned to numbered and lettered cups (A, B or C). Figure 21 is a shortened version of the sensory instrument utilized, and Figure 19 shows the possible combinations tested (C=control and T=treatment). Each combination was tested in triplicate by the 10 panelists. A total of 30 judgements for each combination was obtained.

:Panelist:

This is a triangle test for SMELL AND TASTE
TEXTURE IS NOT A CONTRIBUTING FACTOR IN YOUR DECISION!!

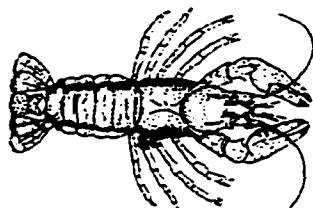
General Procedure:

1. Collect all three sample cups with the same sample numbers, they will differ by being labeled A, B or C.
2. Smell and taste each trio of samples cleansing between **.
3. Mark the sample that was different from the other two. It may be possible for all three samples to taste the same or smell the same. If that is the case, mark no difference.
4. Please indicate if the sample you selected as different is acceptable or not.
5. If the sample you picked was exceptionally good or not so good, please make brief comments describing what you liked about the smell or taste of the sample.
6. Go to the processing room and repeat the procedure for the fillets. Please smell and pick the fillet that appears different.

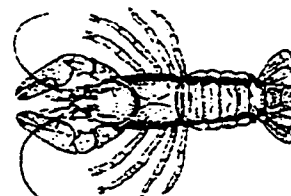
**Smell the sample then taste - spit into the cup, chew a bit of cracker and spit into the cup, and rinse in the cup DO NOT swallow the sample nor the rinse water!

Remember: lunch on Friday.

DATE _____		TASTE PANEL				PANELIST # _____				
	SMELL					TASTE				
Sx	A	B	C	No Diff.	Acceptable Y/N	A	B	C	No Diff.	Acceptable Y/N
Comments on Sx.										



**GO TO THE
PROCESSING
ROOM**



DATE _____		TASTE PANEL				PANELIST # _____				
	SMELL					VISUAL				
Sx	A	B	C	No Diff.	Acceptable Y/N	A	B	C	No Diff.	Acceptable Y/N
Comments on Sx.										

Figure 18. Abbreviated sensory instrument.

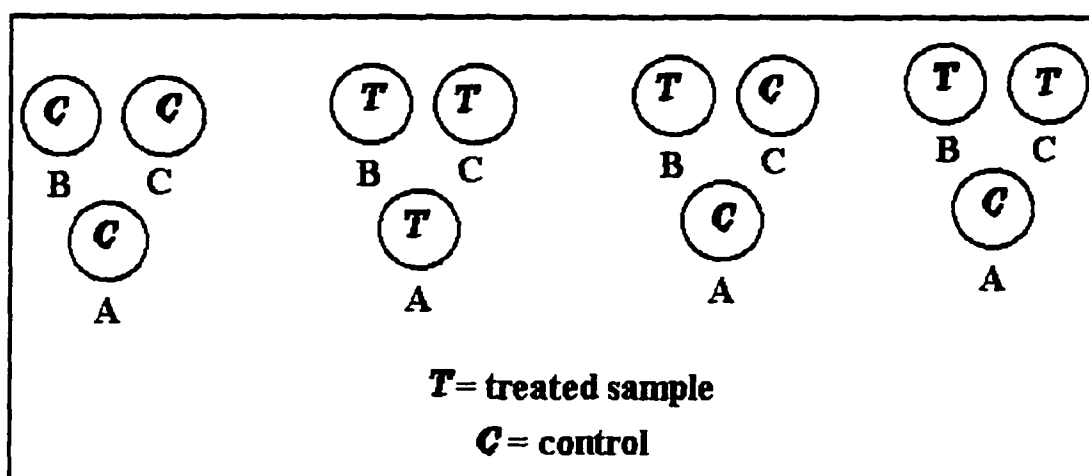


Figure 19. All possible combinations in triangle testing.

Analysis

The number of correct judgements were analyzed for significant differences using the expanded tables given by Roessle et al., (1978). Preference scores were tabulated and analyzed only for those samples in which the panelist correctly identified the difference between samples and general acceptability.

Results and discussion

In comparing treated and untreated cooked fillets, panelists could significantly differentiate ($P < 0.05$) between CCC and TTT arrangements. There were no differences in either TTC or CCT groupings. Figure 20 shows the percent total correct judgements for each triangle arrangement. Fillets could be treated, extending shelf life, and prepared with no

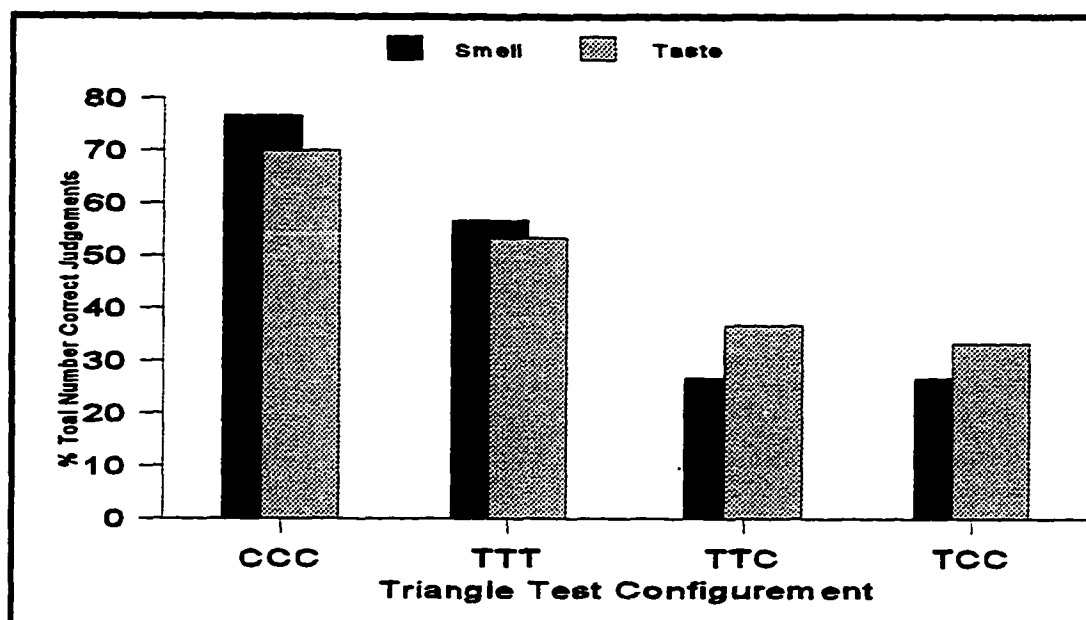


Figure 20. Panelist ability to detect differences in cooked fillets (smell & taste).

distinguishable odor or taste variations. Ninety six percent (96%) of panelist indicated that treated samples were acceptable and preferable to untreated samples.

At 65°C, the 2% acetic acid dipped fillets was effective in reducing microbial counts and suppressing *L. monocytogenes* growth for 6 days (data not published). Treated fillets were significantly able to be distinguished from control fillets, but they were not unacceptable to panelists. Treated and cooked samples were not distinguishable from the control and were acceptable also to panel participants. In comparing raw and dipped fillets,

significant differentiation ($P>0.05$) was found among homogenous groups. Triangle arrangements of CCC and TTT were identified as significantly distinguishable with confidence. This correlates with the Hunter color data. Table 8 summarizes the Hunter color data.

Table 8. Hunter color mean value scores for L, a & b.

Treatment		Mean Values	
Treatment	'L'	'a'	'b'
Raw	45.16 ^a	3.66 ^a	3.36 ^a
Dipped	54.25 ^b	1.80 ^a	3.14 ^a

^{a,b} Different letter in the same column indicate significant difference, Bonferroni T test ($P<0.01$).

Hunter L values were significantly different for raw and treated fillets. The 65°C acetic acid dipped fillets were significantly whiter than their raw counter parts. While a and b values were not significantly different, the obvious whitening could account for the visual differentiation.

Figure 21 shows the percent total number of correct judgements for each triangle arrangement. While no statistical data were gathered, the scent of 'vinegar' was noted on 18% of sensory instruments. Ninety five percent (95%) of panelists reported that

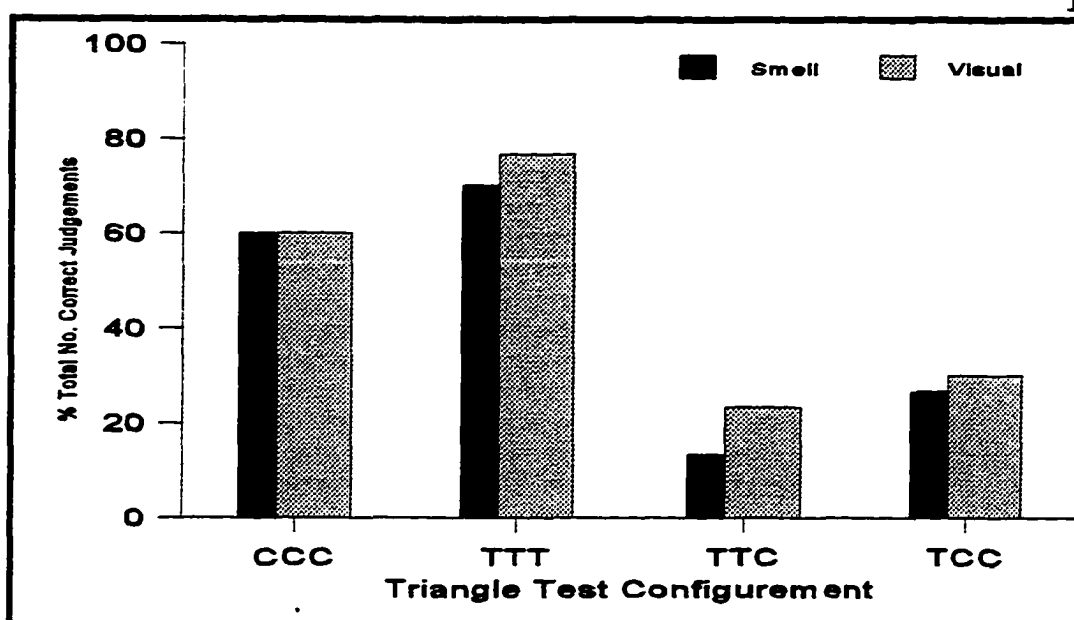


Figure 21. Panelist acceptability in triangle test of raw and treated fillets (visual and smell).

while the fillets were distinguishable, they were also acceptable.

This method of fillet treatment is recommended for restaurant and slaughter house use. The use of hot acetic acid dips were acceptable and could only improve the smell and hygienic status of fish processing plants. Further investigations of treated fillets being involved in a value added product is recommended.

CHAPTER 6
SANITIZER EFFICACY AGAINST *Listeria monocytogenes*
TO STAINLESS STEEL, POLYPROPYLENE AND POLYETHYLENE IN
A BLOOD BIOFILM

Introduction

The growth and attachment of *Listeria monocytogenes* to common seafood processing surfaces in a blood biofilm has not been established and could pose a potential health risk. The objectives of this study were:

- a) investigate the attachment capabilities of *Listeria monocytogenes* strain Scott A to cutlery grade 404 stainless steel, polyethylene knife handles and polypropylene cutting boards in a blood biofilm at ambient temperatures and after 8 hours of abuse time;
- b) to evaluate hot acetic acid (10% solution, 70°C, 5 min. immersion) as a possible GRAS sanitation solution for processing equipment.

The reduction goal for both sanitizing treatments was 3 log cycles. Direct epifluorescent microscopy was utilized to determine the number of reversible and irreversibly attached bacteria to the surface of each material tested. Scanning electron microscopy (SEM) was used to investigate the attachment of *L. monocytogenes* to each surface for reversibly and nonreversibly attached bacteria.

The ability of bacteria to attach and proliferate on a surface has long been established. Veignet in 1947, first established the ability of bacteria to adhere and grow on a glass slide in nearly any environment (Mosteller and Bishop, 1992; Mafu et al., 1990; Spurlock and Zottola, 1991). *Listeria spp.* are widely spread in nature and can survive in a variety of harsh environments.

While *Listeria spp.* have long been documented, negative connotations have been relegated to veterinary medicine, *Listeria spp.* having been deemed a livestock pathogen. In the 1980's *L. monocytogenes* strains emerged as human pathogens for the immunocompromised, elderly and as an abortive agent for pregnant women. *Listeria spp.* ubiquitous presence in nature and ability to grow well in refrigerated temperatures, and blood, invites contamination for foods that undergo hand filleting or trimming. Kerr et al. (1993) has established that *L. monocytogenes* can persist on the finger tips of food workers for up to 3 hours.

The growth and attachment of *L. monocytogenes* to stainless steel (common dairy grade 300 series), iron

drains, polypropylene, rubber, Teflon and glass has been demonstrated with *L. monocytogenes* in culture and in a milk biofilm. *L. monocytogenes* was able to attach to stainless steel, glass, polypropylene and rubber surfaces within 20 minutes of contact. Growth in a blood biofilm has not been established but could pose a potential health risk.

The longer bacteria are allowed to remain attached to a surface, the more resistant they become to removal. Acetic acid and hydrogen peroxide have been shown to reduce microbial growth, but utilization as a sanitizing agent has not been studied. Acetic acid is recognized as a GRAS substance and the establishment as a sanitizing agent would be welcomed by many in the 'organic' movement.

Direct epifluorescent filter technique (DEFT) was used to establish reversibly and irreversibly attached *L. monocytogenes* to tested surfaces. The scanning electron microscope (SEM) was used to visualize the extracellular attachment of glycocalyx of *Listeria monocytogenes* strain Scott A to common processing surfaces before and after treatment with potential sanitizing agents.

Materials and methods

The following information details the materials and procedures utilized in the experiment previously detailed.

Preparation of cultures

Listeria monocytogenes strain Scott A was used in the study. Stock cultures were maintained through monthly transfers on tryptic soy agar (TSA - Difco Laboratories, Detroit, MI) and stored at 23°C. Before each treatment, an inoculum from a stock culture was transferred into a tube of tryptic soy broth (TSB - Difco Laboratories, Detroit, MI) and incubated for 24h at 30°C for three consecutive days. On day three 1ml of inocula was subcultured into 9 ml TSB tubes supplemented with 1% decitrinated sheep blood (TSBB) and allowed to incubate for 12 hours.

Preparation of test surfaces

Randle high grade carbon 404 steel fillet knives, their polypropylene handles and a polyethylene cutting board were used. A number of 3mm by 15 mm strips were cut from each. The chips were cleaned in an ultrasonic scrub chamber with an enzymatic detergent for 30 minutes. The chips were

washed in sterilized distilled water, autoclaved (121°C, 15 min.) in Nalgene screw cap sample cups and held until used.

Soiling procedure

Ten milliliters tubes of TSBB were prepared as previously described. One chip was added per tube per surface type. Tubes were incubated for 8 hours to simulate an abuse scenario, ending of a work shift. After incubation, the chips were removed and rinsed three times for 1 min in peptone water to remove unattached organisms before the surfaces were sanitized.

Sanitizer

Ten milliliter tubes of 10% HAc was prepared. Acetic acid tubes were heated to 70°C. One chip was added per tube per surface type for each solution and treated for 5 minutes. Chips were removed and rinsed 3 times.

Enumeration methodology

Direct epifluorescent filter technique (DEFT) was used to enumerate colony forming units on the three surfaces. Chips were vortexed with 5ml of peptone water for 1 minute to remove the reversibly

attached cells. Each surface was stained with 1ml of DAPI stain for 30 seconds. Sample surfaces were allowed to air dry and mounted on a microscope slide with nonfluorescent immersion oil. Stained colonies were counted using a Nikon Microphot FA microscope.

The average number of clumps per field was multiplied by a microscope factor to estimate the number of bacteria per mm^2 . The MF was calculated by the equation:

$$\text{MF} = \frac{\text{Area of treated surface (mm}^2\text{)}}{\text{Microscope field area (mm}^2\text{) x sample volume (ml)}}$$

Results and discussion

Five fields were examined for fluorescing clumps of cells. Table 9 indicates the number of bacteria per CFU/ mm^2 for each surface.

Table 9. CFU/ mm^2 of 3 processing surfaces after incubation and sanitation.

	Knife Blade	Knife Handle	Cutting Board
Before Sanitizing	$4.2 \times (10^4)$	$6.8 \times (10^7)$	$8.2 \times (10^8)$
After Sanitizing	$> 1.0 \times (10^0)$	$2.1 \times (10^1)$	$3.1 \times (10^1)$

The following figures are photographs of the blade, handle and board in various conditions.

Figure 22 and 23 shows a portion of the fillet knife before and after sanitation. Figures 24 and 25 show

a portion of the fillet board before and after sanitation. Figures 26 and 27 show a portion of the knife hands before and after sanitation. From these photographs and sanitation figures, *Listeria monocytogenes* can easily attach to common surfaces used in hand trimming operations. When large amounts of blood is present, the bacteria persists. By treating the surface with 70°C acetic acid at 10% for 5 minutes, near sterilization is possible with a GRAS substance.

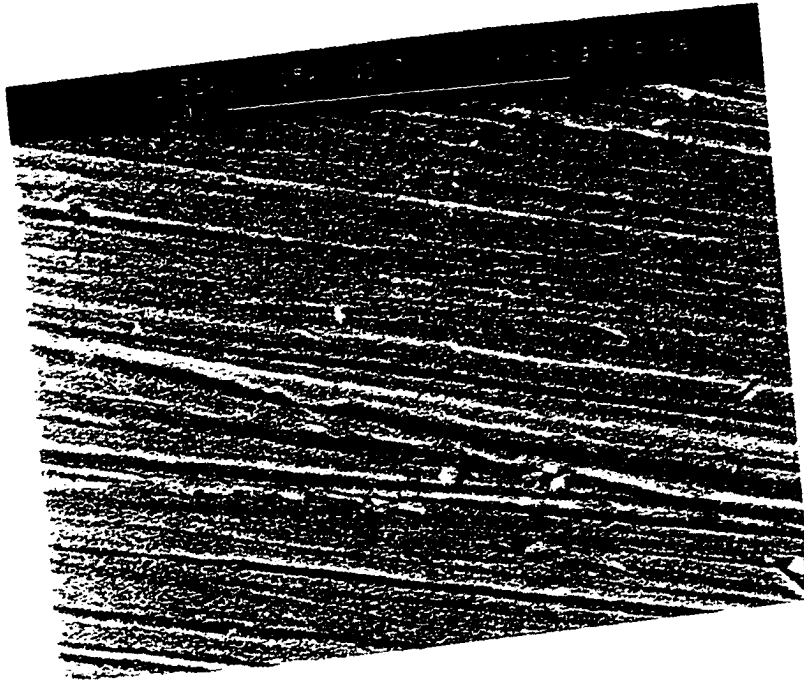


Figure 22. Electron micrograph of sanitized stainless steel.

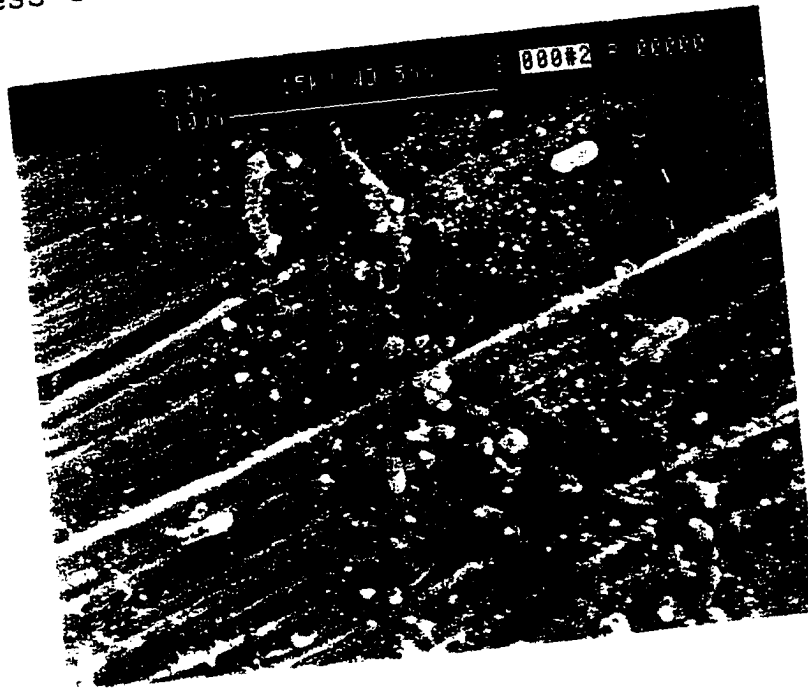


Figure 23. Electron micrograph of stainless steel with *Listeria monocytogenes* biofilm.



Figure 24. Electron micrograph of sanitized polyethylene fillet board.



Figure 25. Electron micrograph of polyethylene fillet board with *Listeria monocytogenes* biofilm.

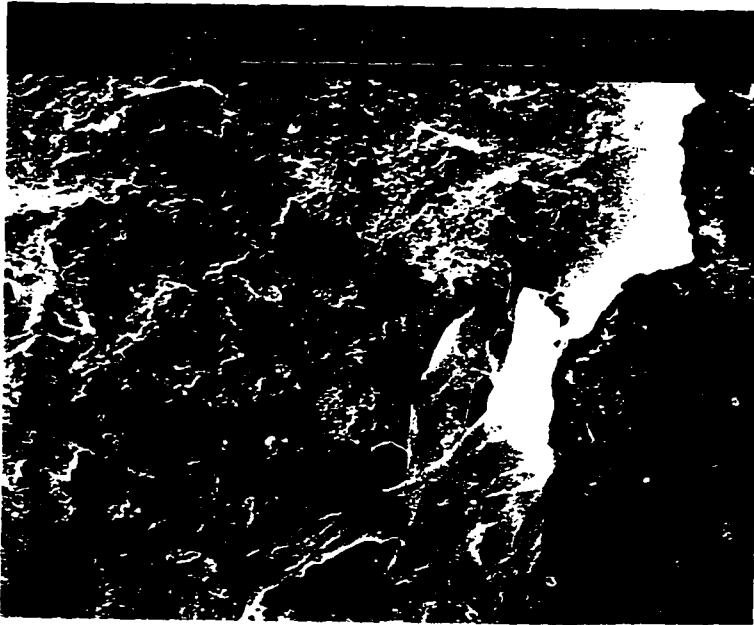


Figure 26. Electron micrograph of sanitized polypropylene knife handle.

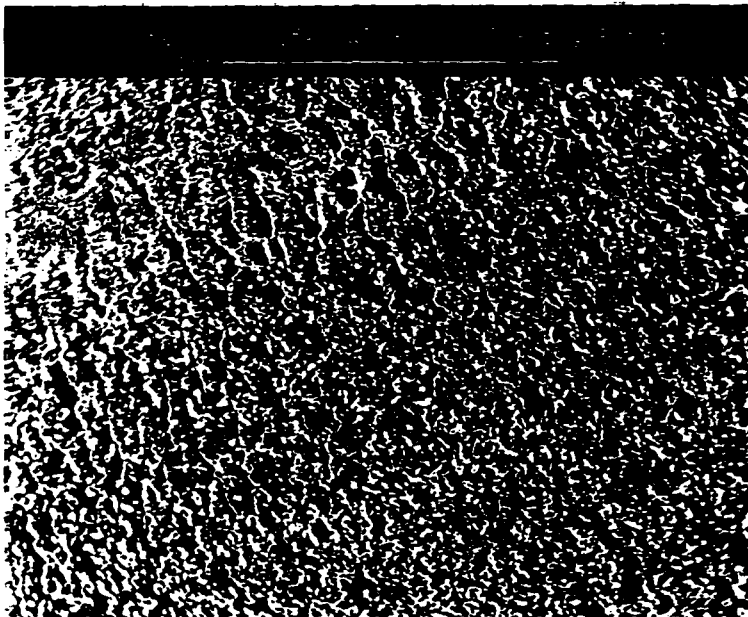


Figure 27. Electron micrograph of polypropylene knife handle with *Listeria monocytogenes* biofilm.

REFERENCES

- Abeyta, C. J. 1983. Bacteriological Quality of fresh seafood products from Seattle retail markets. J. of Food Protection. 46:901.
- Ahamad, N., and Marth, ElH. 1990. Acid-injury of *Listeria monocytogenes*. J. Food Prot. 53:26.
- Ahamad, N., and Marth, E.H. 1989. Behaviour of *L. monocytogenes* at 7, 12, 21 and 35°C in tryptose broth acidified with acetic, citric or lactic acid. J. Food Prot. 52:688.
- Albritton, W.L, Wiggins, G.L., Dewitt, W.E., Feeley, J.C. 1980. *Listeria monocytogenes*. In :Lennette E.H. Manual of clinical microbiology 3rd ed. Washington D.C.: American Society of Microbiology. 139:12.
- Amstutz, H.E. 1980. Listeriosis - *Listeria monocytogenes*, cattle, sheep, goats, veterinary practices. Bovine medicine and surgery. Editor Amstutz, H.E. Santa Barbara, Calif., American Veterinary Publications.
- Anderson, M.E., Marshall, R.T., Stringer, W.C., and Naumann, H.D. 1977. Combined and individual effects of washing and sanitizing on bacterial counts of meat - a model system. J. Food. Prot. 40:688.
- Anonymous, 1984. Listeriosis and death from icecream. Food Che. News 32:26.
- ASTM; 1993 American Society for Testing & Materials. Manual on sensory testing methods. ASTM special technical publication 434. Philadelphia, Pa.
- Ayers, J.C., Ogilvy, W.S. and Stewart, G.F. 1950. Post-mortem changes in stored meats. I. Microorganisms associated with development of slime on eviscerated cut-up poultry. Food Technol. 4:199-205.

- Baldry, M.G.C. 1983. The bactericidal, fungicidal, and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Bacteriol. 54:417.
- Bilio, J.E.; Rosenthal, H. and silverman, C.J. (eds) 1986. Realsim in aquaculture: achievements, constraints, perspectives: Proc. World Conference of Aquaculture, Venice, 21-25, Sept., 1981, European Aquacultuer Society.
- Biocide Intl., Inc. 1994. ECO-Benefits of Oxine-A chlorine dioxide based antimicrobial. Biocide Intl., Inc., Norman, OK.
- Bishop, P.W. 1978 Who introduced the tin can? Nicolas Appert? Peter Durand? Bryan Donkin? Food Technol. 32:4:60-67.
- Blankenship, L.C. 1981. Some characteristics of acid injury and recovery of *Salmonella bareilly* in a model system. J. Food Prot. 44:73.
- Booth, I.R. 1985. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. 49:359-378.
- Braddach, J. 1988. Aquaculture: "Moving from Craft to Industry." Environment, 30:2:7-40.
- Bradshaw, C.M. 1995. Personell communication.
- Brandt, F.I. and Arnold, R.G. 1977. Sensory tests in food product development. Food Prod. Develop. 11:8:56.
- Brock, T.D., Smith, D.W., and Madigan, M.T. 1988. Biology of Microorganisms, 4th ed., Prentice-Hall, Englewood Cliffs, NJ, pp.260-265.
- Brown, E.E. 1988. World Fish Farming - Cultivation and Economics 2nd Ed. AVI publishing, West Port Con.
- Brown, L.R., and Young, J.E. 1990. Feeding the World in the Nineties and Beyond. State of the World, Washington, D.C.: Worldwatch Institute.

- Bruce, G.G. and Broome, C.V. 1989. Listeriosis. JAMA. 261:9:1313.
- Bryan, F.L. 1978 Epidemiology of foodborne diseases transmitted by fish shellfish and marine crustaceans in the U.S. J. of Food Protection, 43:859.
- Busch, L.A. 1971. Human listeriosis in the United States, 1967-1969. J. Infect. Dis. 123:328.
- Busscher, H.J. and Weerkamp, A.H. 1987. Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiol. Rev. 46:165.
- Buttkus, H., Tomlinson, N. 1966. Some aspects of Postmortem changes in fish muscle. Physiology and biochemistry of muscle as a food. eds Briskey, J.J., Cassens, R.g. and Trautman, J.C. University Wisconsin Press.
- CDC (Centers for Disease Control CDC Surveillance Summaries). 1994. MMWR, 32, no ss-1.
- CDC (Centers for Disease Control CDC Surveillance Summaries). 1990. MMWR, 39, no ss-1.
- CDC (Centers for Disease Control CDC Surveillance Summaries). 1987. MMWR, 9, no ss-1.
- CDC (Centers for Disease Control CDC Surveillance Summaries). 1995. MMWR, 3, no ss-1.
- Characklis, W.G. 1984. Biofilm development: A process analysis. In "Microbial Adhesion and aggregation," ed. K.C. Marshall, Springer Verlag, New York.
- Chung, K.T., Dickson, J.S., and Crouse, J.D. 1989. Attachment and proliferation of bacteria on meat. J. Food Prot. 52:173.
- Civille, G.V. 1978. Case studies demonstrating the role of sensory evaluation in product development. Food Technol. 31:11:59.

- CFR (Code of Federal Regulations) 1995. Title 21.
Office of the Federal Register, U.S. Government
Printing Office, Washington, D.D.
- Conner, D.E., Brackett, R.E. and Beuchat, L.R. 1986.
Effect of temperature, sodium chloride and pH on
growth of *Listeria monocytogenes* in cabbage
juice. Appl. Environ. Microbiol. 55:59-63.
- Costerton, J.W., Geesey, G.G., Cheng, K.J. 1978. How
Bacteria Stick. Scientific American. 238:86.
- Criado, M.T., Suarez, B., Ferreiros, C.M. 1994. The
importance of bacterial adhesion in the dairy
industry. Food Technol. 48:2:123.
- Cudjo, K.S. 1988. The effect of lactic acid sprays on
the keeping qualities of meat during storage.
Int. J. Food Microbiol. 7:1.
- Davidson, M.P. and Branen, A.L. 1993. Antimicrobials
in foods. 2nd ed. Marcel Dekker, New York.
- Dawson, E.H. and Dochterman, E.F. 1951. A comparison
of sensory methods of measuring differences in
food qualities. Food Technol. 5:87:79.
- Dellenbarger, L.E., Luzar, E.J., Schupp, A.R.
1988. Household demand for catfish in Louisiana.
Agribusiness. 4:5:493.
- Dember, W.N. 1963. The Psychology of Perception.
Holt, New York.
- Deng, J.C. 1981. Effects of temperature on fish
alkaline protease, protein content and texture
quality. J. of Food Science. 46:62:1981.
- Dickson, J.S. 1990. Survival and growth of *Listeria
monocytogenes* on beef tissue surfaces as
affected by simulated processing conditions. J.
Food Safety. 10:165.
- Difco. 1995. The Difco Manual. Difco Laboratories.
p. 1027, Detroit Michigan.

- Durant, W. 1954. The pre-historic beginning of civilization, in *The Story of Civilization: Our Oriental Heritage*. Simon and Schuster, New York.
- El-Krest, S.E. and Marth, E.H., 1988. Inactivation of *Listeria monocytogenes* by chlorine. *J. Food Prot.* 51:520-524.
- Emswiler, B.S. and Kotula, A.W. 1976. Bactericidal effectiveness of three chlorine sources used in beef carcass washing. *J. Anim. Sci.* 42:1445.
- FAO - 1994. Food and Agriculture Organization of the United Nations - The state of Food and Agriculture. Rome, Italy.
- Fabian, F.W., and Wadsworth, C.K. 1939. Experimental work on lactic acid in preserving pickles and pickle products. II. Preserving value of acetic acid and lactic acid in the presence of sucrose. *Rood Res.* 4:511.
- Farber, J.M. 1991. Current research on *Listeria monocytogenes* in foods: an overview. *J. Food Prot.* 56:7:640-643.
- Farrier, K.T.H. 1979, Who invented the brine bath? *Food Technol* 33:2: 75-77.
- Feinstein, G.R. and Burk, E. M. 1972. Relationship of Texture to pH and collagen content of Yellow Tail Flounder and Cusk. A Research note, *J. Food Science.* 49:1984:299.
- Fennema, O.R. 1985. Food Chemistry. University of Wisconsin. Dekker Pub.
- Fitzgerald, G.A. and Conway, W.S. 1937. Sanitation and quality control in the fishery industry. *Amer. J. Pub. Health.* 27:1094-1101.
- Fleming, D.W., Cochi, S.L., MacDonald, K.L. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New Engl. J. Med.* 312:404-407.

- Fletcher, D.L., Russel, S.M. Walker, J.M., Bailey, J.S. 1993. An evaluation of a rinse procedure using sodium bicarbonate and hydrogen peroxide on the recovery of bacteria from broiler carcasses. Poultry Sci. 72:11:2152.
- Fox, P.F. and Condon J.J. editors; 1985. Food Proteins. Applied science publishers, London, 1982, pp. 270-272.
- Frazier, W.C. and Westhoff, D.C. 1988. "Food Microbiology, 4th ed." (McGraw-Hill Book Co., N.Y.) pp. 243-254.
- Gellin, B.G. Broome, C.V., Hightower, A.W. 1987. Geographic differences in listeriosis in the United States. Abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotheraps. New York, 10:5:87.
- Gould, G.W., and Dring, G.J. 1975. Heat resistance of bacterial sportes to lysozyme and the hydrogen peroxide with agents which rupture disulphide bonds. J. Gen. Microbiol. 33:413-423.
- Hayat, M. 1987. Principles and techniques of electron microscopy. Biological Applications Vol 7. Van Nostrand and Reinhold.
- Hendry, P. 1988. Food and Population: beyond Five Billion. Population Bulletin, 5:1:55
- Hollingworth, Jr. T. A., Kaysner, C.A., Colburn, K. G., Sullivan, J.J., Abeyta, Jr., C., Walker, K.D., Torkelson, Jr., J. D.,Throm, H.R., and Wekell, M.M. 1991. Chemical and microbiological analysis of vacuum-packed, pasteurized flaked imitation crabmeat. J. Food Sci. 56:6:164.
- Honikel, K.O., Fischer, C., Hamid, A. and Hamm, R. 1981. Influence of Postmortem changes in bovine muscle on the water holding capacity of beef- post mortem storage of muscle at -20°C. J. of Food Science. 46:1:86.

- ICMSF (International Commission on Microbiological Specification for Foods) 1980. "Microbial Ecology of Foods. Vol. 1. Factors Affecting Life and Death of Microorganisms." Academic Press, Inc. New York, N.Y.
- ICMF. 1986. "Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Specific Applications," 2nd ed. University of Toronto press, Toronto.
- Irvin, A.D. 1969. The inhibition of *Listeria monocytogenes* by an organism, resembling *Bacillus mycoides*, present in normal silage. Res. Vet. Sci. 10:106-108.
- Israel, D.C. Kahl, K.H. and Pomeroy, R.S. 1993. The effects of relative price perceptions and demographic factors on restaurant catfish consumption. Agribusiness. 5:3:322.
- Jay, J.M. 1992. Microbiological safety of foods. In Modern Food Microbiology 4th ed. Van Nostrand Reinhold, N.Y.
- Jackson, J.M., and Shinn, B.M. 1993. Fundamentals of Food Canning Technology. AVI. Westport, Conn.
- Jay, J.M. Modern Food Microbiology, 1986. Van Nostrand, Reinhold Co. Inc.
- Jenson, R. 1982. A fresh look at marketing seafood. Quick Frozen Foods. Oct. 1983:12.
- Kenji, S., Chiho, O., Kozo, O., Kawabata, M. 1993. Type V collagen in Trout (*Salmo gairdneri*) muscle and its solubility change during chilled storage of muscle. Dept of FDSC and Nut. Kyoto Prefectural University, Shimogamo, Kyoto, Japan, Journal of Agri. and Food Chemistry 39:1222.
- Kerr, K.G., Birkenhead, D., Seal, K., Hawkey, P.M. 1993. Prevalence of *Listeria* spp. on the hands of food workers. J. Food Prot. 56:6:525.

- Kosak, P.H. and Toledo, R.T. 1981. Effects of microbiological decontamination on the storage stability of fresh fish. *J. Food Sci.* 46:1012.
- Leung, C.K., Huang, Y.W., Pancorbo, O.C. 1995, Bacterial pathogens and indicators in catfish and pond environments. *J. Food Prot.*
- Lillard, H.S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. *Poult. Sci.* 59:1761-1766.
- Lillard, H.S., Blankenship, L.C., Dickens, J.A., Craven, S.E., and Schackelfor, S. D. 1987. Effect of acetic acid on the microbiological quality of scalded picked and unpicked broiler carcasses. *J. Food Prot.* 50:112.
- Liston, J. 1980. Microbiology in fishery science. In advanced in Fish Science and Technology. J.J. Connell and Torry Research Station Staff (ed.). Fishing News Books, Ltd., Farnham, Surrey, England.
- Lopes, J.A. 1986. Evaluation of dairy and food plant sanitizers against *Salmonella typhimurium* and *Listeria monocytogenes*. *J. Dairy Sci.* 69:2791.
- Loung, J.H.T., Male, K.B., Masson, C., and Nguyen, A.L. 1992. Hypoxanthine ratio determination in fish extract using capillary electrophoresis and immobilized enzymes. *J. Food Sci.* 57:5:77.
- Lovell, R.T. 1991. Foods from aquaculture. *Food Technol.* 45:87.
- Lovett, J., Wesley, I.V., Vandermaaten, M.J., Bradshaw, J.G., Francis, D.W., Crawford, R.G., Donnelly, C.W. and Messer, J.W. 1989. High-temperature short time pasteurization inactivates *Listeria monocytogenes*. *J. Food Prot.* 54:90.

- Mafu, A.A., Roy, D., Goulet, J. And Magny, P. 1990. Attachment of *Listeria monocytogenes* to stainless steel, glass, poypropylene, and rubber surfaces after short contact times. J. Food Prot. 53:9:742.
- Makukutu, C.A. and Guthrie, R.K. 1986. Survival of *Escherichia coli* in food at hot-holding temperatures. J. Food Prot. 49:7:86.
- McLauchlin, J. Audurier, A. Taylor, A.G. 1986. Aspects of the epidemiology of human *Listeria monocytogenes* infections in Britain, 1967-1984: The use of serotyping and phage typing. J. Med Microbiol. 22:367.
- McVicker, R.J., Dawson, L.E., Mallman, W.L. Walters, S. and Jones, E., 1958. The effect of certain bacterial inhibitors on shelf-life of fresh fryers. Food Tehnol. 12:147-149.
- Miller, T. G. 1993. Living In the Environment. Wadworth England, Thompson Publishing p. 354.
- Mosteller, T.M. and Bishop, J.R. 1992. Sanitizer Efficacy Against Attached Bacteria in a Milk Biofilm. J. Food Prot. 56:1:4-41.
- Muir, J. F. 1981, Management and cost implications in recirculaitns water systems: In Proceedings of the Bio-engineering Symposium for fish culture. (eds Allen, Kinney) Washington D.C. Fish Culture Section, America Fisheries Society, pp.116-127.
- N.A.S. 1994. - National Academy of Sciences, Marine Aquaculture: Opportunities for Growth. Washington, D.C. National Academy Press.
- Norman, J.R. 1963. A history of fish. London England. Benn Ltd.
- Norton, C.F. 1982. Microbiology. University Agusta Main. Addison & Wesley.

- Nunheimer, T.D., and Fabian, F.W. 1940. Influence of organic acids, sugars and sodium chloride upon strains of food poisoning staphylococci. Am. J. Public Health 40:1040.
- Okrend, A.J., Johnston, R.W., and Moran, A.B. 1986. Effect of acetic acid on the death rates at 52°C of *Salmonella newport*, *Salmonella typhimurium* and *Campylobacter jejuni* in poultry scald water. J. Food Prot. 49:500.
- Osthold, W., Skin, H.K., Dresel, W., and Leistner, L. 1984. Improving the storage life of carcasses by treating their surfaces with an acid spray. Fleischwirtsch. 64:828.
- Owusu-Yaw, J., Toth, J.P., Wheeler, W.B. and Wei, C.I. 1990. Mutagenicity and identification of the reaction products of aqueous chlorine or chlorine dioxide with L-Tryptophane. 55:6:1714.
- Pangborn, R.M. 1989. The evolution of sensory science and its interaction with IFT. Food Technol. 43:248.
- Pfaffman, C. , Schlosberg, H., and Cornsweet, J. 1954. Variables affecting differences test. In "Food Acceptance Testing Methodology" Natl. Acad. Sci.-Natl. Res. Counc., Washington, D.C.
- Potter, N.N. 1986. Food Science. AVI publishing, Fourth ed.
- Rawles, D.D., Flick, G., Diallo, A, Croonenberghes, R.; 1995a. Growth of mixed cultures of *Listeria monocytogenes* and *Listeria innocua* in blue crab (*Callinectes sapidus*) meat. J. Food Protection. 58:11:1268-1270.
- Repp, V.E. and McCarthy, W.J. 1989. Metal Work Technology and Practice. Glenco Publishing.
- Rosslar, E.B., Baker, G.A. and Amerine, M.A. 1956. One-tailed and two-tailed test in organoleptic comparisons. Food. Res. 21:117.

- Roessler, R.M., Pangborn, J.L., Sidel and Stone, H. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43:4:940.
- Russell, A.D. 1990. Bacterial spores and chemical sporicidal agents. Clin. Microbiol. Rev. 3:2:99.
- Schummb, W.C., Satterfield, C.N., and Wentworth. R.L. 1955. In Hydrogen Peroxide First Edition (American Chemical Society Monograph Series), New York, Van Nostrand-Reinhold, pp. 813-816.
- Sconce, J.S. 1962. "Chlorine - Its Manufacture, Properties and Uses" J.S. Sconce, Ed. (Malabar, Fl: Robert E. Kreiger Publishing Co., 1962) p.7.
- Schlech, W.F., Lavigne, O.P. Brotolusse, R. 1983. Epidemic listeriosis: Evidence for transmission by food. N. Eng. J. Med 319:823-828.
- Seelinger, H.P.R. and Finger, H. 1976. Listeriosis. P. 333. In J.S. Remington and J.O. Klein (eds.), Infectious diseases of the fetus and newborn infant. W.B. Saunders Co., Philadelphia.
- Seeliger, H.P.R., Jones, D. Listeria in Sneath PHA, Mair, N.S., Sharpe, M.E. 1994 Bergey's Manual of Systematic bacteriology. Baltimore, Williams & Wilkins, vol 2 pp. 1235.
- Shand, G.H. Anwar, H., Kadurugamuwa, J., Brown, M.R.W. Silverman, S.H. and Meling, J. 1985. In vivo evidence that bacteria in urinary tract infections grow under iron restriced conditions. Infect. Immun. 48:35-39.
- Shepard, C.J. and Bromage, N.R. (eds). 1988. Intensive Fish Farming. BSP Professional Books Oxford Press, Oxford England.
- Shineman, T.L. and Harrison, M.A. 1994. Growth of *Listeria monocytogenes* on different muscle tissues. J. Food Prot. 57:12:1057.

- Singh, A., Yu, F., McFeters, G.A. 1990. Rapid detection of chlorine induced bacterial injury by the direct viable count method using image analysis. App. and Environ. Microbiol. 56:2:389-394.
- Smulders, F.J.M., and Woolthus, C.H. J. 1983. Influence of two levels of hygiene in the microbiological condition of veal as a product of two slaughtering/processing sequences. J. Food Prot. 46:1032.
- Sorrells, K.M., Enigl, D.C., and Hatfield, J.R. 1989. Effect of pH, acidulant, time and temperature on the growth and survival of *Listeria monocytogenes*. J. Food Prot. 52:571.
- Snelling, A.M. Kerr, Heritage, J. 1991. The survival of *Listeria monocytogenes* on fingertips and factors affecting elimination of the organism by hand washing and disinfection. 54:4:343.
- SOLVAY, 1960. Hydrogen Peroxide, Solvay Technical and Engineering Service, Bulletin No. H6-0049. Allied Chemical Corp.
- SRAC (Southern Regional Aquaculture Center) #2585, 1993. Recirculating Aquaculture Tank Production Systems: Management of Recirculating Systems.
- Spurlock, A.T. and Zottola, E.A. 1991. Growth and attachment of *Listeria monocytogenes* to cast iron. J. Food Prot. 54:12:925.
- Stewart, G.F and Amerine M. A. 1973. Introduction to Food Science and Technology, New York: Academic Press.
- Stone, H., and Pangborn, R.M. 1968. Intercorrelations of the senses. In "Basic Principles of Sensory Evaluation." ASTM STP 433, pp. 30-46. Am. Soc. Test. Mater., Philadelphia Pennsylvania.

- Surve, A.N., Sherikar, Bhilegaonkar, A.T., Karkare, K.N., 1991. Preservative effect of combinations of acetic acid with lactic or propionic acid on buffalo meat stored at refrigeration temperature. *Meat Sci.* 29:309.
- Trant, A.S., Pangborn, R.M. and Little, A.C. 1981. Potential fallacy of correlating hedonic responses with physical and chemical measurements. *J. Food Sci.* 46:583.
- Trussell, R.P. 1972. The percent unionized ammonium in aqueous ammonia solutions at different pH levels and temperatures. *J. Fish Res. Bd. Canada* 29: 1405.
- USDA - 1995. United States Department of Agriculture Aquaculture (LDP-AQS-1), Situation and Outlook Summaries.
- Van der Marel, G.M., Van Logtestijn, J.G., and Mossel, D.A.A. 1988. Bacteriological quality of broiler carcasses as affected by in-plant lactic acid decontamination. *Int. J. Food Microbiol.* 6:31.
- Viser, I.J.R., Koolmees, P.A., and Bijker, P.G.H. 1988. Microbiological conditions and keeping quality of veal tongues as affected by lactic acid decontamination and vacuum packaging. *J. Food Prot.* 51:208.
- Videla, H.A. 1989. Importancia de los biofilms bacterianos en la corrosión microbiológica de metales y aleaciones. In XII Congreso Nacional de la Sociedad Española de Microbiología. Vol I. p. 319. Spain.
- Webb, N.B. and Howell, B.C., 1975. Effects of additive, processing techniques and frozen storage on the texture of shrimp. *J. of Food Sci.* 40:5:322.

- Weagant, S.D., Sado, P.N., Colburn, K.G., Torkelson, J.D., Stanley, F.A., Krane, M.H., Shield, S.C. and Thayer, C.F. 1988. The incidence of *Listeria* species in frozen seafood products. J. Food Protection. 51:8:655-657.
- Welshimer, H.J., Donker, V.J. 1971. *Listeria monocytogenes* in nature. Appl Microbiol. 21:519.
- White, L., 1976. Food and history, in Food, Man and Science, Walcher, D.N. Kretchmer, N., and Barnett, H.L. New York.
- Whittaker, H.A. and Mohler, B.M. 1912. The sterilization of milk bottles with calcium hypochlorite. Amer. J. Pub. Health. 2:282-287.
- Woolthus, C.H.J., Mossel, D.A.A., VAn Logtestijn, J.G., DeKruiff, J.M., and Smulders, F.J.M. 1984. Microbial decontamination of porcine liver with lactic acid and hot water. J. Food Prot. 46:220.
- Woodward, W.A., and Schucany, W.R. 1977. Combination of a preference pattern with the triangle taste test. Biometrics. 33:31.
- Wyatt, L.E., Ranzell, N.I. and Vanderzant, C. 1979. Occurrence and control of *Salmonella* in freshwater catfish. J. Of Food Sci. 44:79:1067.
- Youssef, H., Kel-Timawy, A.K., Ahmed, S. 1992. Role of aerobic intestinal pathogens of fresh water fish in transmission of human disease. A research note: J. of Food Protection, 55:739.
- Zobell, C.E. 1943. The effect of solid surfaces upon bacterial activity. J. Bacteriol. 46:39-56.

VITA

Michael Adrain Land was born in Alexandria Louisiana, February 11, 1963, during the state's largest snowfall of this century to date. He and the last six generations of his family have lived in north Louisiana and have all been involved in agriculture.

Graduating from Holy Savior Menard High School 1981, he enrolled in Louisiana State University at Alexandria during his high school junior summer and continued there until transferring in 1983 L.S.U.'s main campus in Baton Rouge. After transferring, he graduated in 1984, with a bachelor of science in microbiology. In the fall of 1984, he began attending graduate school at LSU in the Food Science Department. Michael attained his masters of food science degree and certification to teach high school in 1988. He then began teaching at Tara High School then at Baker High School until 1995.

In August 1985, Michael began to pursue his doctorate in Food Science. He is currently a candidate for that degree for the fall semester of 1996.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Michael Adrain Land

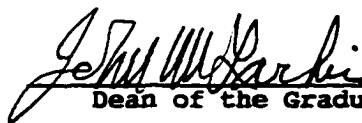
Major Field: Food Science

Title of Dissertation: Chemical Effects on Microbial and Sensory Qualities of Catfish in Planktonic and Dynamic Systems

Approved:

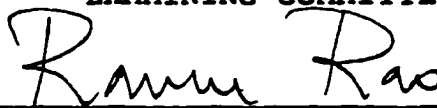


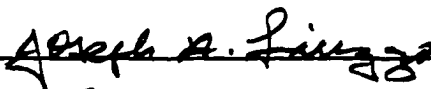
Major Professor and Chairman

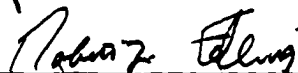


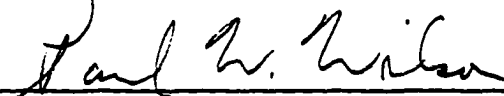
Dean of the Graduate School

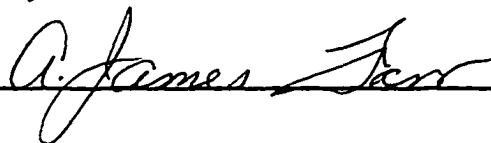
EXAMINING COMMITTEE:











Date of Examination: